Use of alternative wood chips from new botanical species

Their impact on phenolic composition and sensory properties of a rose wine from Touriga Nacional grape variety

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ABSTRACT

The aim of this study was to evaluate the effects that acacia (*Robinia pseudoacacia*), cherry (*Prunus avium*), American and French oak (*Quercus alba* and *Quercus petraea*) wood chips and the evolution of the phenolic composition and sensorial properties of a rose wine made from Touriga Nacional grape variety during a storage period of 20 days.

Chemical analysis were carried out: total phenols, flavonoid and non-flavonoid phenols, chromatic characteristics and tanning power.

At the end of the assay a sensory analysis was performed. Three sensorial parameters group were considered: aspect, aroma and taste.

The results showed some statistical significant differences in most of the phenolic parameters, mainly in the wines without fining. Thus, it was possible to conclude that there are differences between the impact of different botanic species used. In particular, acacia wood chips thus, the results suggest a significant impact on phenolic content of rose wine during the short period of stage, by the use of acacia wood chips.

Concerning to sensorial analysis, two situations could be observed: a slight preference for the wine treated with cherry wood chips – without fining. On the other hand, there was a slight preference for the control wine (without added wood) – after fining.

Key words: wood chips; wine; acacia; cherry; oak; sensory analysis; phenolic composition;

RESUMO

O objetivo deste estudo foi avaliar os efeitos que as aparas de madeira de acácia (*Robinia pseudoacacia*), cerejeira (*Prunus avium*), carvalho Americano e Francês (*Quercus alba* e *Quercus petraea*) têm sobre a evolução da composição fenólica e características sensoriais de um vinho rosé proveniente da casta Touriga Nacional, durante um período de estágio de 20 dias. Em relação à análise química, os parâmetros analisados foram: fenóis totais, fenóis flavonoides e não-flavonoides, aspetos cromáticos e poder tanante.

No final do ensaio foi realizada uma análise sensorial aos vinhos, avaliando três grupos de parâmetros sensoriais: aspeto, aroma e sabor.

Os resultados mostram algumas diferenças estatisticamente significativas na maioria dos parâmetros fenólicos, sobretudo nas amostras dos rosés sem colagem. Assim, pôde concluir-se que há diferenças entre o impacto das diferentes espécies botânicas usadas. Em particular, as aparas de madeira de acácia sugerem um impacto significativo na componente fenólica do vinho rosé em estudo durante o curto período de estágio a que este foi sujeito.

Na análise sensorial, duas situações distintas podem ser observadas: uma ligeira preferência para o vinho que estagiou em contato com aparas de madeira de cerejeira – sem colagem – uma ligeira preferência para o vinho controlo (sem adição de madeira) – após colagem.

Palavras-chave: aparas de madeira; vinho; acácia; cerejeira; carvalho; análise sensorial; composição fenólica;

RESUMO ALARGADO

Nos dias de hoje, a tradição de envelhecer alguns vinhos em barricas de madeira mantém-se. Esta é uma prática recorrente há mais de 2000 anos: a madeira era usada não só para guardar, mas também para transportar vinho e outras bebidas. A espécie mais usada para este fim, é o carvalho, com incidência para as subespécies *Quercus petraea* e *Quercus robur* (os mais comuns nas florestas francesas) e *Quercus alba* (o mais abundante nas florestas americanas).

Durante o processo de envelhecimento do vinho, há um leque de transformações que ocorrem, tais como: alterações químicas e sensoriais (adstringência, aroma e sabor). Os fatores responsáveis por estas modificações são os compostos orgânicos que se extraem da madeira – voláteis e não-voláteis, influenciados pela sua origem botânica, geográfica e respetivo grau de tosta; a incorporação de oxigénio (através dos poros da madeira ou pela atual técnica de micro-oxigenação – no caso do contato com aparas de madeira) e as reações que daí advêm, tornando o vinho mais complexo, estável e com um perfil característico. Contudo, os sistemas de envelhecimento tradicionais (barricas) requerem longos períodos de estágio sob condições muito específicas de temperatura e espaço, que se traduzem em custos elevados e pouco sustentáveis para alguns produtores. A este fator acrescem os custos de investimento na aquisição das barricas. Tais condicionantes têm motivado a implementação de métodos alternativos como a adição de aparas de madeira ao vinho. Assim, há uma simplificação do processo de envelhecimento – no tempo, no espaço, bem como uma redução de custos inerentes ao processo em causa.

Paralelamente ao desenvolvimento de novas técnicas de maturação de vinhos, têm decorrido, nos últimos anos, estudos e ensaios no sentido de avaliar capacidade e utilidade de novas espécies de madeira para o mesmo fim. Acácia (*Robinia pseudoacaia*) e cerejeira (*Prunus avium*) têm sido as mais testadas, ainda que o seu uso não seja ainda legalmente permitido para fins enológicos. Outros trabalhos já desenvolvidos sobre o uso destas madeiras em vinhos tintos (Tavares, 2015) e também em vinhos brancos (Delia, 2016) concluíram que estas novas espécies apresentam um potencial elevado para uso enológico, obtendo bons resultados, sobretudo na análise sensorial.

O objetivo deste estudo centrou-se na avaliação das alterações decorridas ao longo de um período de 20 dias de estágio, na composição fenólica e nas características sensoriais de um vinho rosé, elaborado a partir da casta Touriga Nacional, que estagiou em contacto com aparas de 4 madeiras diferentes com igual nível de tosta (grau médio): acácia (*Robinia pseudoacacia*), cerejeira (*Prunus avium*), carvalho americano (*Quercus alba*) e carvalho francês (*Quercus petraea*). A aplicação das aparas foi realizada a amostras do mesmo vinho rosé – sem colagem (1,5 g/L) e depois do mesmo ter sido submetido à colagem (1,0 g/L).

Em relação à análise química avaliaram-se os seguintes parâmetros: fenóis totais, flavonoides e não-flavonoides; intensidade e tonalidade da cor; antocianinas totais e ionizadas; pigmentos totais e polimerizados e o poder tanante. Os aspetos cromáticos contemplaram também uma análise através das coordenadas obtidas pela aplicação do CIELab.

A avaliação sensorial assentou no estudo dos descritores sensoriais de 3 parâmetros: aspeto, aroma e sabor.

Os resultados obtidos permitem observar, algumas diferenças estatisticamente significativas (entre os vinhos não colados e os vinhos após a colagem) para a maioria dos parâmetros fenólicos estudados.

Em particular, são as aparas de madeira de acácia que demonstram ter maior impacto significativo na avaliação dos parâmetros fenólicos – tanto para as amostras do vinho após colagem como para as amostras do vinho sem colagem. Nas amostras tratadas com a madeira em causa, do vinho rosé sem colagem destacam-se, com valores superiores, os seguintes parâmetros: fenóis não-flavonoides; fenóis flavonoides; fenóis totais; poder tanante.

No parâmetro da apreciação global da análise sensorial é possível observar duas situações distintas: uma ligeira preferência do painel de provadores para o vinho que estagiou com aparas de madeira de cerejeira – nos rosés sem colagem e, por outro lado, o mesmo painel de provadores, sugere uma ligeira preferência para o vinho controlo (sem qualquer adição de aparas de madeira) – nos rosés após colagem.

Os resultados obtidos, ainda que de forma preliminar, sugerem que a aptidão para o envelhecimento de vinhos das novas espécies de madeira – acácia e cerejeira – devem ser levadas em conta, ainda que o seu estudo deva ser mais aprofundado e elaborado. Fatores como o tempo de estágio e a parametrização aplicada a vinhos rosés (neste caso concreto todas as análises efetuadas estão parametrizadas para um vinho tinto) não devem ser descartados, visto que têm uma preponderância nos resultados obtidos bem como na sua interpretação. Contudo, é importante salientar que o estágio de vinhos rosé em madeira (barricas ou aparas) é pouco comum e, por isso, encontra-se pouco desenvolvido. Nesse sentido, são necessárias análises químicas e sensoriais mais detalhadas que desenvolvam e corroborem os potenciais benefícios da maturação de vinhos rosés com madeiras, sobretudo provenientes de novas espécies botânicas.

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1. INTRODUCTION

1.1 Wood – Its role in oenology

The wine industry is an important socioeconomic and cultural sector in many countries worldwide (Fraga *et al*., 2016).

Wood has been used since the time of Imperial Rome in the ageing of wines and other alcoholic beverages. During this period, oak was chosen over other woods, not only for its organoleptic qualities, but also for its physical and mechanical properties, which allow for the construction of a variety of containers, providing them with resistance and porosity, together with the permeability needed during ageing (Jackson, 2000; Fernández de Simón *et al*., 2014a).

The role of oak wood in wine ageing is still crucial in two aspects: the transfer of oak aroma-responsible volatile compounds and astringency-related phenolic compounds to wine; gentle oxidation of certain compounds by atmospheric oxygen (Bozalongo *et al*., 2007). Although ageing conditions, such as the wine cellar temperature and humidity and the length of time in barrel, affect the characteristics of wine (Dubois, 1989).

Also in Portugal, in the XVIII century, there were many cooperages producing barrels to transport water, olive oil, grapes and wine as well as bowls to export (even in a small scale). (Nobre da Veiga, 1954). In the XX century, the use of wood in the ageing of wine was largely recognised due to the physical and chemical properties which lead to modifications in wines (Pontallier *et al*., 1982; Clímaco, 1987; Boidron *et al*., 1988; Dubois, 1989; Chatonnet and Boidron, 1989, 1990; Vivas *et al*., 1991; Clímaco and Duarte, 1992; Chatonnet, 1995; Clímaco and Borralho, 1996; Clímaco *et al*., 1997; Masson *et al*., 1997). In the 60's, Singleton and Draper (1961), promoted the first experiments with the use of wood pieces in the process of wine ageing.

1.2 Traditional wood species

The oak wood used in winemaking is mainly from two sources: American oak (*Q. alba*) and French oak (*Q. robur* and *Q. petraea*) (Cabrita *et al*., 2011). Singleton (1995) studied their chemical composition and demonstrated that they have significantly differences. Besides botanical species, geographic origin also play an important role on the content of the extractive compounds (Prida and Puech, 2006).

Figure 1 - Distribution of *Quercus alba* (A) in North America, *Quercus petraea* (B) and *Quercus pyrenaica* (C) in Europe (www.skyscrapercity.com)

The heartwood used in cooperage has no impact in any physiological function in the tree. Heartwood is resistant to insects and fungi, and is very hard. Its complex structure consists of three main categories of tissue: fibers (support units), parenchyma and radial cells - keeps tissues, as well as conducting vessels (Ribéreau-Gayon *et al*., 2006).

In general, oak wood, contains high levels of polyphenols, namely: ellagitannins, hydrocinamic and hydrobenzoic acids, aldehydes and volatile compounds which vary a lot according to the species, geographic origin, as well as with cooperage procedures (Cadahía and Fernández de Simón, 2004; Cadahía *et al*., 2008).

This kind of wood components transfers to the wine a variety of volatile compounds such as: *cis* and *trans* β-metil-ϒ-octalactone isomers. This wood is well balanced contributing with all these compounds without concealing the primary and secondary wine aromas (Chatonnet and Boidron*,* 1989; Cadahía, 2009).

As can be observed in figure 1, the American oak (*Q. alba)* is largely found in North America. It is one of the species occupying an extensive area and is the most used in cooperage according to its aptitudes.

American oak is quite different from the European oak (*Q. petreaea, Q. faginea, Q. robur, Q. pyrenaica, Q. frainetto)* once it presents a fine compact grain. So, it proves to be more resistant, with more porosity and permeability than the European oak (Cadahía *et al.,* 2008). In addition, chemical composition also differs: however, American oak is richer in volatile compounds and derivatives from lignin degradation and poorer in non-volatile phenolic compounds, mainly tannins (Cadahía *et al.,* 2008) in the comparison with the European oak. It is a very rich wood in aromatic compounds, especially in coconut notes, while the wine is ageing.

French and Portuguese oak woods present ellagitannins contents very similar (Fernández de Simón *et al*., 1998; Jordão *et al.,* 2007; Alañón *et al*., 2011). However, (Fernández de Simón *et al*., 2006; Jordão *et al*., 2007), concluded that *Quercus pyrenaica* wood shows higher levels in ellagitannins than *Quercus petraea*, especially high levels of castalagin and vescalagin.

Table 1- Subspecies of *Quercus,* adapted from Franco (1971)

Subspecies			
Sclerophyllodrys Schwarz	***Cerris Orsted***	***Quercus***	***Erytrobalanus Orsted***
Q. coccifera L.	*Q. suber* L.	Q. robur L.	*Q. rubra* L.
Q. rotundifolia Lam.	*Q. cerris* L.	*Q. pyrenaica* Willd.	*Q. coccinea* Muenchh.
Q.ilex L.		*Q. canariensis* Willd.	*Q. palustris*
Q. chrysolepis L.		*Q. faginea* Lam.	*Q. veluntina* Lam.
		Q. lusitanica Lam.	*Q. falcate* Michx.
		Q. pubescens Willd.	*Q. pallustris* Muenchh.
		Q. bicolor Willd.	
		Q. stellata Wangenh.	
		Q. lyrata Walt.	
		Q. michauxii Nutt.	
		Q. pontica KL Koch	
		Q. macrocarpa Michx.	
		Q. dentate Thunberg.	
		Q. alba L.	

1.3 Some new wood species for oenological use

Besides the traditional wood species used in wine ageing, other species are also increasingly regarded for this use. Recently, the most common species tested in different experiments are false acacia (*Robinia pseudoacacia*) and cherry (*Prunus avium*). Also chestnut (*Castanea sativa*) has been tested in different experiments. This wood (chestnut) had the highest total content of low molecular weight phenolic compounds, followed by the Portuguese oaks and the French oaks, whereas the American oak had the lowest content of these compounds (Canas *et al.,* 2005). This is one of the reasons that their use can be interesting as well as a good option for winemakers to choose wisely the woods for ageing of wines.

Due to their lower costs for local use in certain productions like ciders or balsamic vinegars, they are largely well considered and accepted (Chinnici *et al*., 2011).

1.3.1 Acacia

Some characteristics of acacia wood (*Robinia pseudoacacia*) - like presence of flavonoids and compounds with the β-resorcylic structure, and the absence of hydrolysable tannins, should be taken into account when considering its use in cooperage (Sanz *et al*., 2012a). The use of acacia wood for ageing vinegars is increasing due to the air transfer efficiency that favours a good acetification rate and to its effects on the phenolic composition evolution and sensory quality of vinegars (Sanz *et al*., 2011).

Concerning the hardness of acacia wood (*Robinia pseudoacacia*), its low porosity and low release of tannins into wine (Citron, 2005; De Rosso *et al*., 2009a) polyphenol content from this wood was confirmed to be low, consequently only a small part of tannins will be transferred to the wine (De Rosso *et al*., 2009a). The analysis of the extracts of *Robinia pseudoacacia* heartwood revealed the presence of a wide variety of polyphenols (although in small amounts) which belong to very different chemical families (Sanz *et al*., 2011). Its grain showed to be coarse and irregular. Its texture proved to be strong and inequal (Carvalho, 1997).

However, if toasted barrels are used, the acacia wood will contribute condensed tannins as well as the other flavonoids (Sanz *et al*., 2011). The most interesting low molecular weight phenolic compound regarding the organoleptic characteristics is vanillin, which provides larger quantities when compared with other wood species (Fernández de Simón *et al*., 2014b).

Wines aged in acacia barrels were more abundant in simple volatile phenol compounds while wines aged in oak barrel had more oak lactones (Kozlovic *et al*., 2010). No significant differences were detected when the same wine is aged in oak or acacia barrels – suggesting the same physical-mechanical properties like porosity for the two kinds of barrels (Sanz *et al*., 2012b).

Acacia is also characterized for having significant quantities of benzenic aldehydes (De Rosso *et al*., 2009a). This wood, when compared with oak, cherry and chestnut, may be considered as the richest in lignin derivates and carbohydrates.

1.3.2 Cherry

Cherry wood is characterized for having low density, porosity with medium or low hardness with distinct heartwood - brown or redish - and also regular and undefined (Carvalho, 1997).

Although there are few studies concerning the influence of cherry wood in wine ageing process, some information can already be obtained after some studies in this area.

Thus Chinnici *et al*., (2011) claimed that if compared with oak, cherry wood promoted a faster evolution of wine constitutive phenols. This evolution was characterized by a greater reduction of wine flavanols and flavonols, the formers being involved in condensation phenomena able to stabilize wine colour (Chinnici *et al*., 2015).

When compared with oak wood, cherry heartwood contains not only relatively large amounts of flavonoid compounds but also high concentrations of some volatile compounds (Sanz *et al*., 2010).

Some authors reported that comparing with oak wood, cherry wood is richer in lignin derivates and poorer in lipid and carbohydrates derivates (Fernández de Simón *et al*., 2009; Culleré *et al*., 2013; Fernández de Simón *et al*., 2014b).

On the other hand, cherry wood after toasting, showed a low level of polyphenols and hydrolysable tannins, which proves that this wood is not like oak wood used in cooperage. The difference is due to oak wood having high concentration of hydrolysable tannins (Cadahía and Fernández de Simón, 2004).

Several aromatic compounds were found in cherry wood extracts, although in low abundance, and a high amount of trimethoxyphenol. These extracts were characterized by very low amounts of eugenol and methoxyeugenol, and the lowest fatty acid contents (De Rosso *et al*., 2009a).

Cherry wood is also characterized by high porosity and oxygen permeation, and is usually used for short ageing times (Citron, 2005; De Rosso *et al*., 2009a) once this type of wood is the most oxidative environment, when compared with other woods used in cooperage (De Rosso *et al*., 2009b).

1.4 Extractable compounds

During wine ageing a transference of different phenolic and volatile compounds from oak into wine occurs. This process depends on the potential extractable compounds initially present in the wood (Del Álamo Sanza *et al.,* 2004a).

The extraction of the above compounds is influenced mainly by some factors: the oak species, the geographical origin and the sylvicultural treatment of the tree (Canas *et al.,* 2000), but the most important factor is the wood processing in cooperage (Spillman *et al.,* 2004). These organic compounds, mainly phenolics, influence the color, astringency, bitterness, oxidation level and clarity of wines (Garcia *et al.,* 2012).

Flavour is made up of volatile compounds, which are responsible for smell, and non-volatile compounds, which cause taste and different sensations, such as sweetness, acidity, bitterness and saltiness (Espitia-López *et al.,* 2015).

Among the extractable substances, volatile and non-volatile, the most oenologically important are ellagitannins, gallic acid, ellagic acid, various aromatic compounds and aldehydes.

Some authors (Chatonnet *et al*., 1990; Cadahía *et al*., 2001) suggested that the structural characteristics of oak could influence the concentration of wood extractive compounds.

1.4.1 Volatile compounds

One of the most important aspects of ageing wines in oak concerns the aromatic compounds that are extracted. They make a significant contribution to the richness and complexity of the bouquet, as well as improving the flavour (Ribéreau-Gayon *et al*., 2006).

Volatile compounds are associated to aroma of the wine. However, the nature of the compound is related not with the chemistry but to the fragrance (Jackson, 2000).

Oak also contains a high level of volatile compounds that have a great impact on aroma of wood-matured wines (Ribéreau-Gayon *et al*., 2006). Heartwood provides a lot of volatile compounds which contribute to the aroma and flavour of aged wines (Sanz *et al*., 2012a).

Many volatile compounds in natural oak wood have been described but only a few of them have significant impact on the sensory characteristic of wines (Guchu *et al.,* 2006).

Oak wood compounds contributing to wine aroma are mainly: furfural (dried fruit, burned almonds), eugenol (spices, cloves and smoke character), vanillin (vanilla character) and syringaldehyde (also related to vanilla character) (Bozalongo *et al*., 2007).

Some phenolic compounds are found in fresh wood (such as vanillin and eugenol), but most of them are originate and develop as a consequence of the hydroalcoholysis of lignin, which occurs due to the burning of the barrel (Flanzy, 2003). These compounds influence the colour, astringency, bitterness, oxidation level and clarity of wines and are also involved in the changes taking place during wine ageing (Singleton, 1992). Toasting increases their quantities and leads to the formation of other ones. Furanic aldehydes, caused by carbohydrate degradation and responsible for the "toasty" aromas, and lignin degradation compounds (vanillin, syringaldehyde, guaiacol) tend to be formed during the toasting process (Guchu *et al.,* 2006).

In contrast, much higher amounts of non-volatile constituents must be absorbed to influence taste or aspect (Jackson, 2000).

Table 2 - Some volatile compounds extracted from wood, their description and sensorial perception levels present in wines ageing in wood. a Boidron et al. (1988); bChatonnet et al. (1992); c Chatonnet (1995); d Wilkinson et al. (2004). Adapted from Jordão (2005).

Compounds	Perception level (mg/L)	Sensorial descriptors
Furfural	15a, b	Caramel, almond
***Metil*-5-furfural**	16 – 45a, b	Caramel, acetone, bitter almond
***Trans*-β-metil-γ-octalactone**	0,067 – 0,11[b, c]	Green nut, wood
***Cis*-β-metil-γ-octalactone**	0,046 – 0,79[c, d]	Wood, vegetal
Vanilin	0,032 – 0,065[b]	Vanilla
Guaiacol	0,02 – 0,075[a, b]	Smoke
Eugenol	0,015 – 0,5[a]	Cloves
Etil-4-guaiacol	0,047 – 0,14[a, b]	Smoke, wood
Phenol	5,5 – 25[b]	Spices
m-Cresol	0,065 – 0,38[a]	Pharmacy
Syringaldehyde	50a	Pepper, spices

1.4.2 Non – Volatile compounds

Quantitatively, non-volatile phenolics are the most important group of oak extractives (Jackson, 2000).

Oak tannins appear to indirectly play a role on colour stability, by promoting anthocyanin polymerization with condensed tannins (Vivas and Glories, 1996). These molecules are soluble in hydroalcoholic environment - that is why they are gradually extracted by wine during its ageing in oak barrels (Michel *et al*., 2011). Their level in oak wood used to make barrels depends on the processing of wood in cooperage namely the the type and length of drying and toasting process (Jordão *et al.,* 2007; Michel *et al*., 2011).

The hydrolysable tannins (gallotannins and ellagitannins), the main polyphenols released from wood make up the most significant subgroup of oak non-flavonoids (Jackson, 2000). They play a very important role in wine affinity. Polysaccharides confer astringency, structure and colour stabilization to the product (Puech *et al.,* 1999; Ribéreau-Gayon *et al*., 2000).

Ellagitannins are the most important ones, since they can represent up to 10% in dry weight of oak heartwood. Once in the wine, they are slowly but continuously transformed through condensation, hydrolysis, and oxidation reactions. This gives rise to the formation of other compounds, as their ethyl derivatives and the flavanol-ellagitannins (Jourdes *et al.*, 2011). Consequently, their levels in aged wines will be much lower than those of other compounds (Fernández de Simón *et al.,* 2014a).

Some authors (Chira and Teissedre, 2013b), observed that ellagitannins levels in a model wine solution explained 45% of the total astringency variance. They also prevent the development of a brick-yellow colour by preventing the oxidation of phenolic compounds (Vivas and Glories, 1996).

Vescalagin and castalagin are largely predominant in oak wood species. They represent between 40% and 60% by weight of the ellagitannins in *Quercus petraea* and *robur* heartwoods (Michel *et al*., 2011).

The effect of toasting on the non-volatile wood constituents, more specifically to ellagitannins, may be regarded as being rather questionable. Ellagitannins are large molecular weight polyphenols, which have been claimed to possess a series of beneficial biological activities, including antioxidant, estrogenic, anti-inflammatory and antimicrobial (Garcia-Muñoz and Vaillant, 2014).

1.5 Main factors that affect wood composition

The demands placed on wine cooperage require that the wood used in barrel construction possess very specific properties (Jackson, 2000). When the use of wood is considered in wine ageing some factors should be taken in account such as: geographical origin, botanical wood species, heating treatment during barrel manufacture (heating or toasting), dimension of the chips, ageing time or the addition of oxygen during this period (Eiriz *et al*., 2007).

1.5.1 Type of grain

The wood must be straight-grained, that is possess vessels and fibers running parallel to the length of the trunk, with no undulating growth patterns or vessel intertwining. In addition, the wood should exhibit both strength and resilience (Jackson, 2000). Stave wood is commonly classified considering the notion of "grain", described as fine, medium or coarse (Jordão *et al*., 2007).

Grain is a technical term used in cooperage for the wood ring width. Coarse of grains are due to the conditions of the cambial growth of the tree. Thus, one year's growth includes one layer of spring wood and one layer of summer wood, forming one growth ring. The succession of growth rings, year after year, leads to growth of the trunk, with the width of the growth rings representing the wood grain. According to this theory, wood can be divided into different grains according to the average width of growth rings (Jordão *et al*., 2007).

Some authors (Chatonnet *et al*., 1990; Cadahía *et al*., 2001) suggested that the structural characteristics of oak could influence the concentration of wood extractive compounds. Vivas (1995a) reported a correlation between wood ring width and ellagitannin concentration in unidentified oak species.

1.5.2 Seasoning: natural and artificial

Natural seasoning influences the aromatic profile of wood. Oak wood dried and seasoned outside undergoes the action of microorganisms, rain, sun, and air, what eliminates a portion of the ellagitannins through hydrolysis, leaching, and oxidation. Opposite to artificial seasoning, natural seasoning has predominantly a positive effect on volatile composition and sensorial characteristics of oak wood which directly influence sensorial characteristics of the (Matricardi and Waterhouse, 1999; Vivas *et al.,* 2004; Cadahía *et al*., 2007; Fernández de Simon *et al*., 2010a).

Natural seasoning can also result in changes in the concentration of several oak extractable components with aromatic impact in wine sensorial characteristic, such as aroma profile. The degradation products of lignin, such as eugenol, vanillin, and syringaldehyde, have been variously noted: the outer portions of the staves can increase or decrease (Jackson, 2000). Natural seasoning is an operation that takes several years. There is a relationship between the time and the thickness - 24 or 36 months for 21mm or 28 mm staves, respectively. This length of time is necessary to obtain wood that is properly suited to the ageing and improvement of wine (Taransaud, 1976). The seasoning of the wood is imperative once the use of green wood (70% of humidity) contains an excess of phenolic compounds which transmit a bitter taste as is the case in ellagic tannins and cumarines, as well as a very low concentration in phenolic compounds (Fernández de Simon *et al.*, 2010b). Thus, the adequate final value of humidity percentage of the wood to be used should be about 15% (Jordão *et al*., 2006).

Seasoning takes place in the open air, in large level spaces. It has been estimated that oak seasons at a rate of about 10 mm per year. In fact, intense dehydration takes place during the first 10 months. This is followed by a period when the wood matures, thus improving its physical and aromatic qualities. Seasoning is, however, heterogeneous, depending on the position of the wood in the pile (Doussot *et al*., 2002).

Natural seasoning leads to an increase in the concentrations of various aromatic compounds: eugenol, syringic and vanillic aldehydes produced by the breakdown of lignin, as well as both isomers of *β*-methyl-*γ* -octalactone, with a higher proportion of the more odoriferous *cis* form. The effect of microorganisms on these odoriferous compounds is can be considered responsible to a varying extent for reducing concentrations of these substances (Ribéreau-Gayon *et al*., 2006).

Artificial seasoning consists of keeping split oak in a ventilated drying oven at 40–60◦C for approximately 1 month. This technique considerably reduces seasoning time, without altering the oak's physical properties. It also eliminates the financial investment tied up in a wood seasoning lot (Ribéreau-Gayon *et al*., 2006). However, green wood must be seasoned gradually, to avoid shrinkage cracks.

Nevertheless, this type of seasoning has certain effects on the development of the compounds in the oak. Most of the reactions described that take place in natural seasoning process do not occur under these conditions (Fernández de Simón *et al*., 2010a).

Compared to naturally seasoned wood, oven-dried wood has a higher content of astringent tannins and bitter coumarins. It contains less eugenol, vanillin and β-methyl-octalactone, with a majority of the less odoriferous *trans* isomer (Spillman *et al.,* 2004).

It is well known that natural seasoning is better for the quality of barrel aged wines. A combined process that has been tested may make it possible to benefit from the main advantages of each technique (Francis *et al*., 1992)**.** Natural seasoning is used for in-depth modifications ('maturing' stage) and artificial seasoning for rapid, homogeneous dehydration (Cadahía *et al*., 2007). The use of both techniques can result in a certain degree of success (Ribéreau-Gayon *et al*., 2006).

1.5.3 Heating

Barrel quality depends on successful toasting. This has a major impact on the later development of the wine during ageing, as well as the organoleptic characteristics it acquires (Sanz *et al.,* 2011).

Heating facilitates the bending of the staves to produce the characteristic barrel shape. It affects the plasticity of the lignin, but has little impact on the glucide polymers (cellulose and hemicellulose), as these compounds are protected by the humidity they absorb. The combination of heat and humidity makes it possible to bend barrels into shape without breaking the staves (Ribéreau-Gayon *et al*., 2006).

1.5.4 Toasting

The process of barrel toasting probably has the main influence on the chemical compounds of the wood, which are susceptible to migrating into wine during their ageing, affecting their organoleptic properties (Chatonnet *et al*., 1990; Francis *et al.,*1992; Pérez-Coello *et al*., 2000). Is a crucial practice used in cooperage that causes deep changes in the chemical composition of oak wood, which could affect its antioxidant capacity (Jordão *et al.,* 2012).

Heating oak also leads to the formation of volatile compounds that may have several origins (Cadahía *et al*., 2001). Is furthermore responsible for the breakdown of ellagitannins, present in decreasing concentrations as toasting intensity increases (Bozalongo *et al*., 2007).

Toasting is of irrefutable importance to the generation of new volatile substances that favorably enrich the aromatic profile of wines (Chira and Teissedre, 2013a; Fan *et al*., 2006; Michel *et al*., 2013) because it causes extensive thermal degradation of the wood biopolymers lignin and cellulose/hemicellulose and formation of compounds with a spectrum of flavor tones, such as honey/caramel (furfural), vanilla (vanillin, syringaldehyde), smoky/spicy (eugenol, guaiacol) (Kanakaki *et al*., 2015). When comparing forest origin and toasting, the results showed that the toasting affects more considerably furanic, guaiacol, vanillin and ellagitannin concentration (Chira and Teissedre, 2015).

The toasting process causes an important decrease in ellagitannin content, antioxidant capacity, and scavenging activity. Otherwise, with the toasting process, the total phenolic content and the concentration of ellagic acid increase (Jordão *et al.,* 2012).

Toasting drastically enhances the gain in volatile compounds arising from the thermal degradation of oak wood (Cutzach *et al*., 1997; Chatonnet *et al*., 1999; Doussot *et al*., 2002).

Benzoic/cinnamic aldehyde ratios were determined to distinguish between products aged in chestnut or oak wood, and in toasted or untoasted barrels (Canas *et al*., 2004). Furan and pyran derivatives are compounds with a toasty caramel aroma. They are formed as a consequence of the heating treatment carried out in barrel-making (Cutzach *et al*., 1997).

All toasting operations affect the surface and internal structure of the oak (Chatonnet, 1991). Usually, there are three levels of toasting: light, medium and heavy.

Light toast indicates a toasting time of approximately 5 minutes, with a surface temperature between 120 and 180◦C. The inside of the barrel has a spongy aspect, due to modification of the lignins and hemicelluloses, while the cellulose structure remains intact (Ribéreau-Gayon *et al*., 2006).

The lowest level of heating has been found to reduce the level of vescalagin by 73% and castalagin by 46% in the surface layer wood (Moutounet *et al.,* 1989).

Medium toast corresponds to a toasting time of approximately 10 minutes, producing a surface temperature of approximately 200◦C. The parietal surface components disappear by fusion.

Heavy toast corresponds to a toasting time of more than 15 minutes, resulting in a surface temperature of approximately 230◦C. The cell structure is considerably disorganized, while the surface is blistered and covered with tiny cracks (Ribéreau-Gayon *et al*., 2006).

Following heavy toasting, the increase in methyl-octalactones contributes a hint of coconut, but this is generally masked by the overall aromatic complexity (Moutounet *et al.,* 1989).

In general, the concentration of eugenol, vanillin and oak lactones in oak wood can be increased by medium or heavy toast levels (Chatonnet *et al*., 1989; Gimenez-Martinez *et al.,* 1996; Cadahía *et al*., 2001). When toasting progresses from light to heavy the oak aroma becomes more complex.

This aroma is initially characterized by toasty and vanilla overtones from the furanic and phenol aldehydes, as well as smoky, spicy and roasted odours from the volatile phenols (Chatonnet, 1991).

According to Guchu *et al.,(*2006), the toasting of the oak wood had a greater influence on the sensory characteristics of wines than the type of the oak wood used.

1.6 Wine ageing in wood

Since the end of last century, the need to reduce production costs, as well as the advance in technology, has led to develop new methodologies in the ageing in cellar for different beverages (Fernández de Simón *et al.*, 2014b).

Wood ageing is a well-established practice in the production of high quality wines. This technique can promote the migration of several compounds from the wood into the wine which may positively influence the complexity, intensity of flavour and aroma (Jaruta *et al.,* 2005).

Oak wood compounds contributing to wine aroma are mainly furfural (dried fruit, burned almonds), eugenol (spices, cloves and smoke character), vanillin (vanilla character) and syringaldehyde (also related to vanilla character) (Bozalongo *et al*., 2007).

During the ageing period of wine, anthocyanins undergo a series of processes that affect directly the wine colour. These processes take place mainly during oxidative breeding period or storage period in wood, rather than during the reduction time (Del Álamo Sanza and Nevares Domínguez, 2006). In order to obtain elegant and well-balanced wines, the ageing process should be carefully carried out (Garcia *et al.,* 2012).

1.6.1 Barrels

Concerning to the sensorial characteristics, the maturation of wine in wood barrels modifies its smell and taste and reduces its astringency; hence, organoleptic properties of wine are improved (Garcia *et al*., 2012).

Although ageing conditions, such as the wine cellar temperature and humidity and the ageing of time in barrel, affecting the characteristics of wine (Dubois, 1989; Towey and Waterhouse, 1996a, 1996b), the most important factor is the raw material of the barrel – oak – and its treatment (Towey and Waterhouse, 1996b; Miller and Howell, 1992), as these two factors determine the wood compounds extracted into wine (Arapitsas *et al*., 2004).

The materials used to make barrel stoppers and the method of barrel closure also play a role for the level of oxygen in the wine. Precipitation of salts, colloids and polyphenols, and CO_2 loss are fundamental for wine limpidity (De Rosso *et al*., 2009a).

During ageing, the development of wine's aromas in barrels is linked to phenolic compounds, suggesting that there is an interaction between the two (Escalona *et al*., 2002).

The production of volatile substances during fermentation is increased when wood is present and is also influenced by the type and quantity of oak used (Aiken and Noble, 1984;

Waterhouse and Towey, 1994). This is probably the reason why barrel-fermented wines show better integration of wood and wine (Schahinger and Rankine, 1995).

Oxygen permeation through the wood favours redox processes and the formation of new and stable anthocyanin and tannin derivatives, with the consequent colour stabilization of red wines, and a loss of astringency (Ribéreau-Gayon *et al*., 1998). In addition, some authors (Del Alamo Sanza and Nevares Domínguez, 2006), concluded that wine aged in barrels had the lowest loss of anthocyanins.

Barrel ageing also affects wine colour. Oak barrels are porous recipients that allow oxygen to enter continuously (Perez-Prieto *et al*., 2003). On the other hand, the prolonged use of a barrel causes a progressive colmatation of the wood pores and a decrease in the wine oxygen content (Vivas, 1995).

After the barrel has been used three to five times, the quantity of the aroma compounds that can be extracted and the quantity of dissolved oxygen in wines will be very close to that of wines stored in tanks (Vivas, 1995). This means that barrels have a limited lifetime. (Rodríguez-Rodríguez *et al*., 2001).

Other disadvantages are the longer ageing time, the higher cost, the short period they can be used, the need of much space in winery (Rodríguez-Rodríguez *et al*., 2001), the contamination risk by undesirable microorganisms (Kyraleou *et al*., 2015) such as *Brettanomyces*, which may produce sensorial significant concentrations of ethyl phenols, compounds with unpleasant medicinal and horsy aromas (Suarez *et al*., 2007) as well as the loss of wine due to evaporation (Kyraleou *et al*., 2015).

1.6.2 Wood Alternatives products: Chips

The benefits of wood ageing are recognized, but it is also known to be very expensive. As an alternative, the use of staves or chips to provide oak characters to wines is becoming quite successful (Cabrita *et al.,* 2011).

Wood alternatives are, however, growingly considered by winemakers who can take advantage of the reduced economic investment, the fast completion of the process, and the comparable results in terms of sensory impact (Natali *et al.,* 2006).

In 2005, the European Union authorized the use of oak wood pieces in winemaking, and consequently, the addition of oak chips has been generalized (Jordão *et al.,* 2012).

Cheaper alternatives have been developed to simplify the ageing process while ensuring that wood components are liberated into the wine. One of these techniques consists in adding small pieces (chips) to the wine (Casassa *et al.*, 2008; Rodriguez-Rodriguez and Gomez-Plaza, 2011). Thus, conferring wood characteristics to the wine with a faster and more simply way (Fernández de Simón *et al.,* 2010b).

Wood pieces may be used in different forms. They could be added to the grape must in the crusher an also in the press with the marc, or placed into tanks of finished wine (Gómez García-Carpintero *et al.,* 2011).

Chips can be added to wine fermented in stainless steel tanks, to obtain wines like those fermented in barrels, but with better manipulation conditions (better temperature control, also able for larger amounts, better control of hygiene) (Pérez-Coello *et al.,* 2000; Rodríguez-Rodríguez *et al*., 2011).

Oak chips are also cheap materials which can be obtained from wood scrap wastes produced during barrel manufacturing, and are prepared using traditional methods in cooperage and subjecting them to boiling in water and toasting (Bozalongo *et al*., 2007).

The size of commercial oak wood pieces can be also expected to have an important effect on the concentration of ellagitannins and antioxidant capacity of the wines matured in contact with these alternative oak wood products (Jordão *et al.,* 2012).

Nowadays, a great variety of oak wood pieces for this purpose can be found on the market: chips, cubes, powder, shavings, granulates, blocks or segments and even staves. The quantity of added wood, time of contact between wood and wine, piece size, the way the wood is used and many other aspects influence the sensorial and chemical characteristics of the wines produced (Fernández de Simón *et al.*, 2014b).

This shortcoming of oak chip technology has now been overcome with the advent of micro-oxygenation technology (Moutounet *et al.,* 2001), which introduces minute and controlled quantities of oxygen into maturing wine (Young *et al.,* 2010).

The above technique presents a valid alternative for production of wine with woody character. (Bozalongo *et al.,* 2007).

Wine ageing employing oak chips should embrace a compromise between the volatiles deriving from toasting and the preservation of wood ellagitannins, to enrich wines not only with flavour substances, but also with polyphenols (Kanakaki *et al.,* 2015).

The use of oak chips provides aromas of oak, vanilla, and spices, increasing and improving the sensory properties of aged wines. Furthermore, compared with barrel ageing, intrinsic fruity attributes of grape varieties used are well expressed in the wines aged with oak chips (Schumacher *et al.,* 2013).

Extraction of phenolic compounds was greater with chips compared to the barrel; therefore, in less time wine aged with chips could be equal to barrel-aged wine (Espitia-López *et al.,* 2015).

The wine aged in contact with oak chips experienced faster ageing, loss of certain compounds and the variety of oak used is more noticeable than the wine aged in barrels. (Gutiérrez Afonso, 2002). Therefore, the ageing treatment by means of toasted chips must be dealt carefully by winemakers to guarantee the success of the ageing process (Schumacher *et al.,* 2013).

In summary, in wines aged with chips, the maximum concentration of the volatile compounds present in wood can be reached in only one month (Rodríguez-Bencomo *et al.,* 2009).

It could therefore be claimed that wines aged with chips and those aged in barrels can happily coexist on the market, with each type of wine targeting different consumer sectors (Pérez-Magariño *et al.,* 2011).

1.7 Vinification methods of rose wines

Rose wines production accounted for nearly 10% of total world wine production in 2011 (Blot and Couderc, 2013). However, the consumption of rose wines represents a growing trend in the wine market, which is becoming an important part of the total wine market. In the last few years it has largely increasing in countries such as France and United States of America (Blot and Couderc, 2013). Their composition varies widely between the light and fruity white wines - low in phenolic compounds - and the coloured red wines, with a high content in anthocyanins and tannins (Di Stefano, 2010). The aroma of rose wines is mainly due to the compounds formed and released during fermentation (Marais, 1983; Baumes *et al*., 1986).

Rose wine colour is directly influenced by grape variety. Colour is an important factor determining rose wine character and quality. This property has an important effect on overall acceptability by the consumer (Mazza, 1995; Revilla-*et al.,* 1999).

The colour of rose and red wine is basically due to the extraction of anthocyanin pigments from grape skins during vinification. In the production of rose wines maceration times are short. This brief contact with the skins and stems gives the wine its light pink colour (Kelebek *et al.,* 2007).

Those wines have some similarities to red wines: they are often made from red grape varieties and contain a small quantity of anthocyanins and tannins. They are also refreshing, like many white wines. For this reason, white winemaking techniques are also used in their production. It is important to monitor grape ripeness. Grapes should not be overripe - potential alcohol not exceeding 12% by volume. Softer roses need slightly higher potential alcohol levels and lower acidity (López-Vélez *et al.*, 2003).

Some studies showed that, although a longer maceration time is positively correlated to the colour stability of wine over time. However, a lengthener of the maceration is not favourable to enrich the wine with pleasant fruity and flowery aroma compounds (Suriano *et al.,* 2015).

Rose wine may also be a by-product of drawing juice out of vats to enhance the concentration of the remaining red wine (Ribéreau-Gayon *et al*., 2000).

1.7.1 Direct Pressing

This widely used method consists of using white winemaking techniques on red grapes, directly pressing fresh grapes. A certain degree of maceration is necessary to obtain colour. It is done directly in the press cage, while the crushed grapes are being drained. This method therefore does not require as quick an extraction as white winemaking methods (Ribéreau-Gayon *et al*., 2000).

Pressing methods have an important effect on wine quality: increasing the pressure, the total phenolic compound extraction increases as well (Salinas *et al.*, 2003). The press juice from the last pressing cycle may be eliminated: the reason is it supplies more tannins than anthocyanins, thus providing a vegetal taste (Revilla *et al.,* 1999).

Anthocyanin fixation results in a slight colour decrease but it is brighter and less sensitive to oxidation. Immediately after extraction, the juice should be protected from oxidation by sulfiting. To preserve the aromas of rose wines they should be kept at relatively low temperatures (Suriano *et al.,* 2015).

1.7.2 Skin contact or drawing off

In order to obtain deeper-coloured, fuller-bodied rose wines the skins and seeds must be kept in contact with the juice for a short time. This method permits to extract more anthocyanins and tannins. However, excessive skin contact may result in too much colour, accompanied by marked astringency and bitterness (Salinas *et al.*, 2003).

The juice should be kept in contact with the grape solids in the press for short periods of time (2–20 hours): this technique is known as pre-fermentation skin contact. This process may also take place in a vat for a longer period (10–36 hours). Then some of the juice is drawn off and fermented as a rose wine (Ribéreau-Gayon *et al*., 2000).

In white and rose winemaking maceration, temperatures lower than 20°C are usually employed (Salinas *et al*., 2003). Higher temperatures may produce musts with a more intense colour but less colour stability due to a greater propensity to oxidation (Salinas *et al*., 2005).

Moreover, since both colour and aromas are fragile and frequently fleeting during storage, the rose wine must be able to keep its qualities over time (Salinas *et al*., 2005). Pre-fermentation maceration of crushed grapes under controlled conditions is a well-known procedure. The aim is to enhance the presence of volatile and coloured compounds, extracted from berry skin into wines (Arnold and Noble, 1979; Marais and Rapp, 1988; Cabaroglu *et al.*,1997).

In both cases, grapes are transferred either directly to a pneumatic press with the drains closed, or to a vat. After a variable period on the skins (2–36 hours), the juice is separated from the solids, either by pressing or by drawing off all or part of the liquid from the vat (Salinas *et al*., 2005).

In certain cases, only part of the juice (10–20%) may be drawn off from the vat. Once a certain volume of juice has been drawn, it is refilled with crushed grapes. This technique is not only to produce a rose wine but also to enhance the phenolic content and colour of the remaining red wine, by increasing the solid/liquid ratio in the vat (Blot and Couderc, 2013).

1.7.3 Carbonic Maceration

Traditionally, methods related to carbonic maceration have been and are still used in Beaujolais (France), Rioja (Spain), and Georgia. In Georgia, grape clusters are poured into large, earthen-ware vessels (kvevri), half-buried in the ground, and sealed with cork. In Rioja, the grape harvest was also poured in vinaira (lagares) made of various materials, usually stone. In these practices, there is no flushing out of the air with carbon dioxide (CO2).
After a period of rapid adoption in many regions in Europe, North and South America, Australia, yujkand Japan (Flanzy *et al.,* 1987), many producers have abandoned carbonic maceration or use it with only a fraction of the harvest. This relates primarily to the high costs associated with manual harvesting – the grapes must arrive at the cellar with the least possible physical damage. Anaeorobic metabolism (AM) occurs only when the anatomical integrity of the berries is maintained. This limits the use of mechanical harvesting to varieties with tough skins. In addition, must flowing out of damaged or crushed fruit is rapidly fermented by yeasts present on berry skin. Under these situations, AM development is very limited or non-existent, depending on the degree to which the integrity of the berry is altered (Jackson, 2011).

The carbonic maceration winemaking technique can be divided into four steps: healthy grape clusters in a tank whose atmosphere is already mainly composed of carbon dioxide (CO2); the ‘‘maceration–fermentation’’ step, where a changing proportion of the grapes are submerged either in a gaseous or in a liquid phase; the free escape or pumping of the juice and pressing

of the grapes that respectively generate the slightly fermented juice and press-run juice; and the second fermentation stage, during which yeast and malolactic fermentation occur (if the latter is desired) (Barre, 1969).
After the completion of fermentation, racking, clarifying, and stabilization, the wine may be bottled (if early commercialization is desired), or matured for several years (if ageing is desired) (Jackson, 2011).

1.8 The grape variety: Touriga Nacional

Few would dispute that the Touriga Nacional is Portugal's finest red grape variety, deserving a place right up at the top of the world league of grapes, along with the likes of Pinot Noir, Cabernet Sauvignon and Nebbiolo. Though northern in origin, it has spread right across the country - you will find it down south in the Algarve and the Alentejo, out west in the Ribatejo/Tejo and Setubal regions, successfully competing with the local Baga grape in Bairrada, and way out mid-Atlantic in the Azores (www.winesofportugal.info).

This grape variety is widely considered to be the most favoured and preferred grape variety in the Douro. It is highly vigorous, has low yields and produces small dark blue berries high in phenolic compounds which impart a deep colour to the wine (Böhm, 2007).

The grapes are thick-skinned, and those skins are rich in colour and tannins, giving excellent structure and ageing capacity. But it also has wonderful, intense flavours, at the same time floral and fruity - ripe blackcurrants, cherry, violet, raspberries - with complex hints also of herbs and liquorice (www.winesofportugal.info).

The wines from this grape variety is highlighted by the descriptors orthonatal and retro-nasal procedures related to Fruity and Floral (Vilela *et al*., 2016).

Its most highly valued properties cannot develop in insufficient sunlight or under extreme drought stress (Böhm, 2007).

Although this grape variety easily adapts to all kinds of soil, it needs high insolation and heat adjusting to most rootstock according to the fertility and soil water availability (www.ivv.min-agricultura.pt).

The wines coming from Touriga Nacional grape variety present a very high ageing capacity, with special aptitude for ageing in wood (Böhm, 2007).

Figure 2 - Grape cluster and leaf of Touriga Nacional grape variety (www.winesofportugal.info)

2. AIM OF THE STUDY

The importance of wood in the ageing and storage process of the wine is, nowadays, indisputable.

Many previous studies proved that oak is the most used species to this purpose. However, the chemical differences that subspecies present and consequently the influence they have on wines, have been evident in different experimental works. The intensive use of oak wood in cooperage as well as the need to innovate, have been the guides to find new alternatives – from barrel use to new wood species. In addition, the use of wood chips in rose winemaking it is also not common.

Acacia and cherry have been studied and experimented in order to understand their aptitude in wine ageing. Their use in oenology is not yet allowed.

Thus, this work intends to evaluate the effects of wood chips from acacia (*Robinia pseudoacacia*), cherry (*Prunus avium*), American oak (*Quercus alba*) and French oak (*Quercus petraea*) in the evolution of the phenolic composition and sensorial properties of a rose wine ageing in contact of these wood chips during 20 days.

3. MATERIAL AND METHODS

3.1 Wine and wood materials: experimental conditions

The wine used in this experiment was a rose wine made entirely from Touriga Nacional grape variety, harvested during the vintage 2015 by Casa da Passarella Winery located in Dão region, following pre-fermentation skin contact method.

Pre-fermentative maceration during 8h; Three fining agents were used with the sequence below: 50 g/hl PVPP; 1,5 g/hl of Isinglass after 12h; 30g/hl of Bentonite. Before the experiment the general physic-chemical wine characteristics were: alcohol content 13,5 % (v/v); total acidity 6,12 g/L (expressed in tartaric acid); volatile acidity 0,26 g/L (expressed in acetic acid); pH 3,23; free corrected sulfur dioxide 38 mg/L; total corrected sulfur dioxide 146 mg/L.

The wood chips used were: Acacia (*Robinia pseudoacacia*) wood chips from SAI company; Cherry (*Prunus avium*), American (*Quercus alba*) and French (*Quercus petraea*) oak wood chips from AEB Bioquímica Portuguesa company. All the wood chips presented a medium toasting level and a particle dimension of 8 mm (medium size). The different wood chip species were added to rose wine samples: before and after fining.

Figure 3 - Examples of wood chips of different species: American Oak (1), Acacia (2), Cherry (3), French Oak (4)

In this experimental work the impact of 4 different wood chips was tested in a rose wine. Moreover, the same wine was used in two different samples: before and after fining.

A total of 10 assays were made in this study, using 2,8 L of rose wine in each assay: rose wine without fining + 1,5 g/L of wood chips for all the wood species and a control wine (without wood chips addition); rose wine after fining + 1 g/L of wood chips for all the wood species and a control wine (without wood chips addition).

Wine samples had been ageing at cellar temperature (15°C – 18°C) for a period of 20 days and stirred twice a week till the third week, with minimum contact with oxygen. The wines were stored in 2,8 L glass carboys. A sample of each wine was collected after 20 days for analysis. A sample of the control wine was also collected before starting the experiment to perform the analysis.

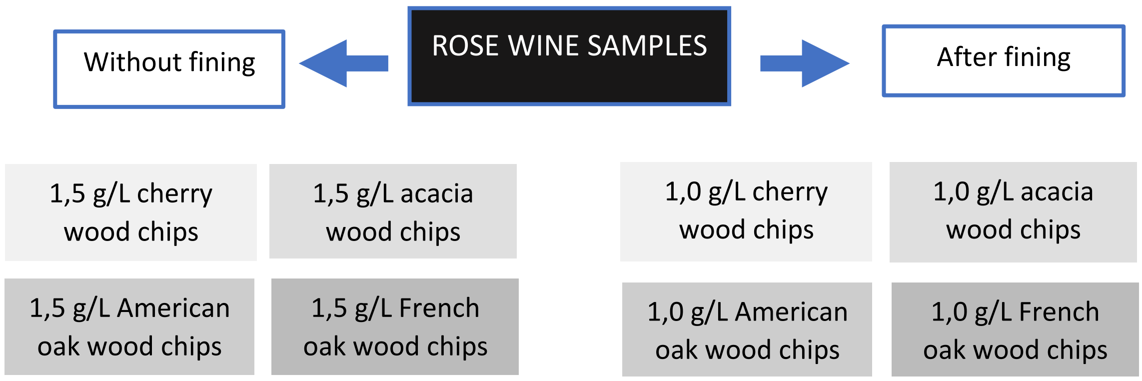

Figure 4 - Wood chip species and quantities distribution by wine samples without and after fining

3.2 Chemical analysis

Before this experiment, the wine used was analysed for pH, total and volatile acidity, alcohol level, total and free SO_2 content using the analytical methods recommended by OIV (1990). All the following determinations, except CIELab method (for the colour estimation in wines) that was performed in Polytechnic Institute of Viseu, were carried out at the laboratory Ferreira Lapa (Enology Sector) of Instituto Superior de Agronomia. All the analysis, except CIELab method (that was made in duplicate), were made in triplicate.

3.2.1 Total phenols; non-flavonoid and flavonoid phenols

The methodology used consisted in the measurement of the absorbency at wavelength 280 nm (A280) of the diluted wine sample (Somers and Evans, 1977). The final value is multiplied by 20 because of the previous dilution.

$$Total\ Phenols = A_{280}\ x\ 20$$

The quantification of non-flavonoid phenols (mainly phenolic acids) in a wine is based on the determination of the phenolic content before and after the precipitation of the flavonoids through the reaction with the formaldehyde, as under certain conditions (low pH, room temperature, darkness) non-flavonoid phenols do not precipitate. After 72 hours, a dilution with distilled water (1:10) is carried out and the absorbance is read at 280 nm at the spectrophotometer (Kramling and Singleton, 1969).

The final value is multiplied by 10 because of the previous dilution. On the other hand, flavonoid phenols come out from the difference between total phenols and non-flavonoid phenols. All the results were expressed in Abs Units.

3.2.2 Colour parameters: intensity, hue and CIELab method

Usually colour intensity in wines is evaluated directly through the sum of the absorbance values of the wines at 420, 520, 620 nm (OIV, 2009).

$$Abs = A_{420} + A_{520} + A_{620}$$

The parameter colour hue was calculated by the quotient between the absorbance at 420nm and 520nm (Sudraud, 1958).

$$Abs = \frac{A_{420}}{A_{520}}$$

Chromatic characteristics, scanned from a range of 380-770 nm to collect data to determine L*(%) (lightness), a* (redness), b* (yellowness) coordinates using CIELab method, were determined according to OIV (1994) recommendations. Chroma $\left(C^* = \sqrt{(a^*)^2 + (b^*)^2}\right)$ value was also determined.

To distinguish more accurately the colour, the difference, which numerically quantifies the colour perception difference between two samples (Negueruela *et al.,* 1995), was calculated using the following equation:

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

Colour differences can be distinguished by the human eye when the difference between ΔE* values are greater than two units (Spagna *et al.,* 1996). All analysis were carried out in duplicate.

3.2.3 Anthocyanins

Some anthocyanins are present in wine in colourless forms, the ones responsible for the colour are the ionized anthocyanins. The total anthocyanin content and ionized anthocyanin content were determined using the methodology developed by Somers and Evans (1977), by spectrophotometry in a cuvette with a 10mm path length, and the expressions to the calculations are:

$$Total\ Anthocyanins\ \left(\frac{mg}{L}\right) = 20\ x\ (A_{520}^{HCl} - \frac{5}{3}A_{520}^{SO_2})$$

$$Ionized\ Anthocyanins\ \left(\frac{mg}{L}\right) = 20\ x\ (A_{520} - A_{520}^{SO_2})$$

$$Degree\ of\ Ionised\ Anthocyanins\ (\%) = \frac{A_{520} - A_{520}^{SO_2}}{A_{520}^{HCl} - \frac{5}{3}A_{520}^{SO_2}}\ x\ 100$$

3.2.4 Total and polymeric pigments

The total pigments are the sum of a wide variety of molecules such as phenolic compounds (such as flavonoids), anthocyanins and associations between them.

The total pigments are calculated through the methodology described by Boulton (1999) based on the following expression:

$$Total\ Pigments\ (u.a) = A_{520}^{HCl}\ x\ 101$$

The polymeric molecules comprehend associations of anthocyanins and tannins, polymeric tannins such as procyanidins. These substances contribute to the colour, body and astringency of wine. The polymeric pigments are essentially polymerized anthocyanins and polymers of anthocyanins and condensed tannins. In young wines, the red colour is also intensified by those anthocyanin polymers. This determination is carried out by the methodology described by Boulton (1999) and the result is calculated through the expressions:

$$Polymeric\ Pigments\ (u.a) = A_{520}^{SO_2}\ x\ 10$$

$$Polymerization\ Index\ (\%) \frac{A_{520}^{SO_2}}{A_{520}^{HCl}} x100$$

3.2.5 Tanning Power

The tanning power stands for the expression of the tannicity of a wine, namely, the capacity that some phenolic compounds as tannins should interact with proteins, influencing the astringent character of the wine at taste.

As described by De Freitas and Mateus (2001) the method includes a dilution 1:50 with a hydroalcoholic solution (12% v/v, pH=3,2 and T=20°C), followed by the reading at the turbidimeter (d_0). Then, 8 mL of the previous dilution and 300 µL of BSA (Bovine Serum Albumin; 0,8 g/L concentrated) are put in a tube and, after an agitation and 45 minutes in the darkness, a second reading is carried out at the turbidimeter (d_1). The final value (NTU/mL) is calculated from:

$$Tanning\ Power\ \left({}^{NTU}/_{mL}\right) = \frac{d_1 - d_0}{0{,}08}$$

3.3 Sensory analysis

The sensory evaluation of the collected wine samples was carried out after 20 ageing days in Polytechnic Institute of Viseu, by a panel composed by eight expert judges trained in quantitative sensory descriptive analysis of wines. The sensory analysis was performed at 18-20°C (room temperature) in a sensory analysis room with individual cabins for each expert. In the session about 30 mL of each sample were presented labelled in a three-digit random code corresponding to a specific treatment. All the evaluated wine samples were served in random order to each judge.

The wines were evaluated using different descriptors for aspect (colour and limpidity), aroma (persistency, intensity, quality, fruity, woody, floral, vegetal and equilibrium), taste (acidity, sweetness, bitterness, persistency, astringency and equilibrium) and global appreciation. The experts scored each sensory attribute on a 1 to 5-point scale (1=absence; 2=little intensity; 3=moderate intensity; 4=intense; 5=high intensity) for each characteristic according to their sensory knowledge, training and experience.

3.4 Statistical analysis

In order to study the influence of four different wood chips species in a rose wine (without and after fining) an analysis of variance (ANOVA) and comparison of treatment means (Tukey's test) was performed using Microsoft Excel and Statistix Version 9.0 (for Tukey's test), in the sensory analysis as well as in all the chemical analysis.

Results were displayed as mean values of three simultaneous assays in all the chemical methods. Statistical significance (at $p<0,05$) of the differences between mean values was assessed by Tukey's test.

4. RESULTS AND DISCUSSION

4.1 Phenolic Parameters

4.1.1 Total phenols; non-flavonoids and flavonoid phenols

Figure 5 shows the evolution of total phenols content of a rose wine samples aged with different wood chips species after 20 ageing days. Rose wine after fining samples evidence values close to each other, resulting on less significant differences, as the wines treated with oak wood chips show.

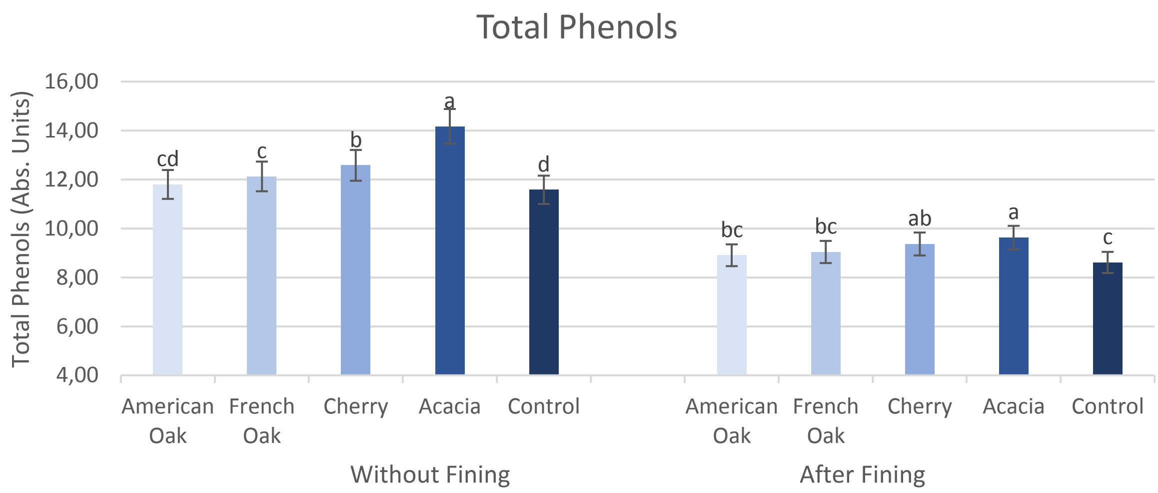

Figure 5 - Concentrations of total phenols after 20 days of storage period of a rose wine (without and after fining) in contact with different wood chips species. Values with different letters indicate that statistically significant differences were detected with Tukey's test (ρ<0,05)

The graphic also shows that rose wine without fining samples have higher total phenol values, when compared with rose wine after fining samples. This difference can be explained by the fining process, once after those application, fining agents can promote the removal of phenolic compounds. Some authors defend that polyphenol extraction as a function of the amount of chips added to the liquid medium was shown to follow a specific pattern, described by an exponential equation (Psarra *et al*., 2015).

On the other hand, (Sanz *et al*., 2010) concluded that toasted cherry wood showed low levels of total polyphenols and hydrolysable tannins.

Figure 6 depicts non-flavonoid parameter values (all the values are expressed in abs. units); Wines from acacia barrels showed flavonoid and non-flavonoid compounds extracted from toasted (barrel staves) acacia wood that were not present in the wines from oak wood (Sanz *et al*., 2012b).

Wine aged with acacia chips showed the highest values during the storage period in both cases, though it is a slight superiority

Regarding rose wines after fining, samples treated with oak wood chips differ from each other but the French oak does not differ significantly from control sample.

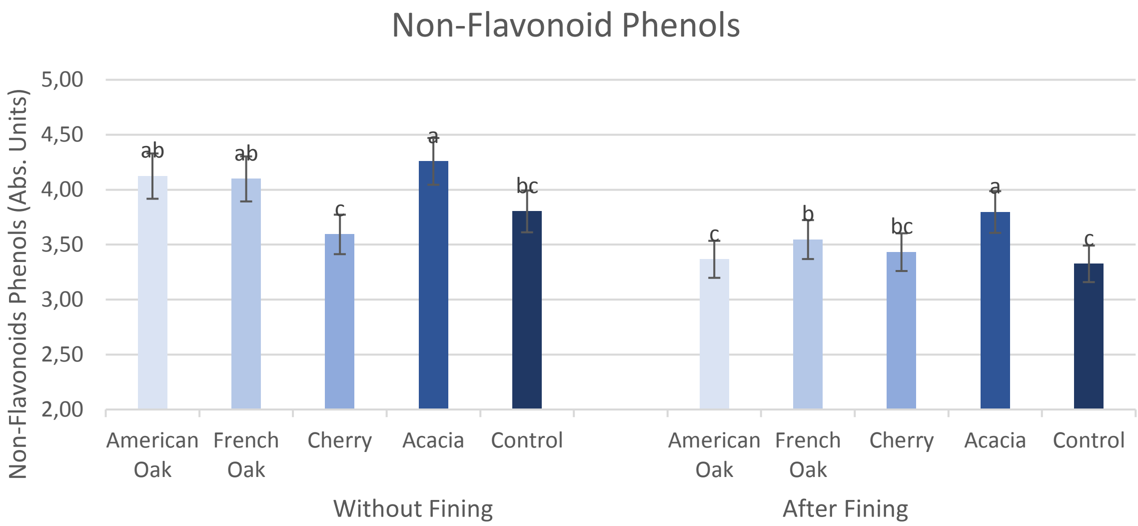

Figure 6- Concentrations of non-flavonoid phenols after 20 days of storage period of a rose wine (without and after fining) in contact with different wood chips species. Values with different letters indicate that statistically significant differences were detected with Tukey's test *(*ρ <0,05)

Figure 7 shows the values of flavonoid phenols in the wines are due to the transfer of compounds from the wood to the wine (Jindra and Gallender, 1987). Similarities can be found between total phenols and flavonoid phenols. Acacia and cherry are again significantly different from oak, with similar values on wine samples where the wood chip samples were added fining and unfining wines. In rose wine without fining, American and French oak are not significantly different from control wine, resulting the same significance letter (c), suggesting no influence by the wood chips during the 20 storage days. Some studies (Sanz *et al*., 2011) concluded that only acacia and cherry heartwood showed flavonoids among their phenolic constituents.

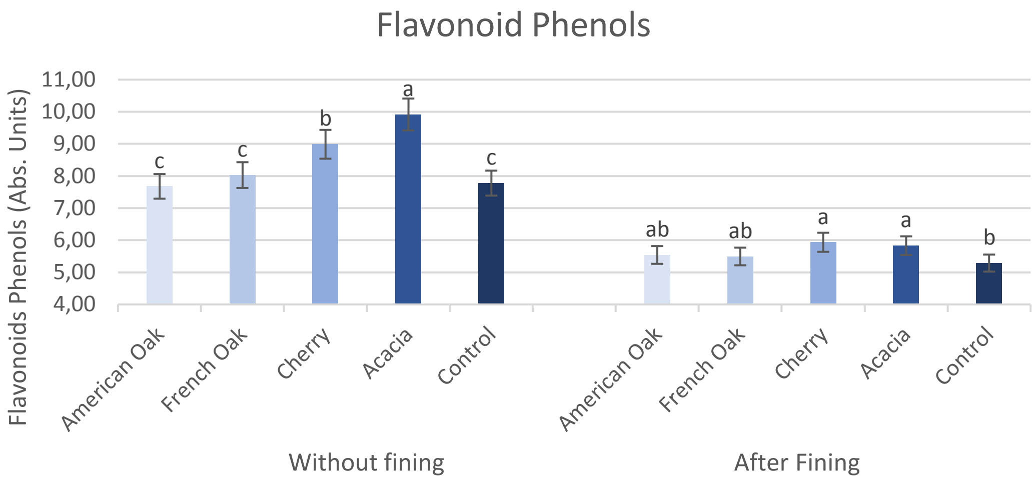

Figure 7 - Concentrations of flavonoid phenols after 20 days of storage period of a rose wine (without and after fining) in contact with different wood chips species. Values with different letters indicate that statistically significant differences were detected with Tukey's test *(ρ <0,05)*

Nevertheless, acacia stands from the other wood chips on rose wine without fining, with a value near 10 (abs. units). Consequently (Sanz *et al*., 2011) proved that flavonoids were the major phenolic compounds in seasoned and toasted acacia heartwood. On rose wines after fining, acacia and cherry showed similar values.

4.2 Tanning Power

Tanning power denotes the tannins capacity to react with proteins predicting the astringency perception of a wine (De Freitas and Mateus, 2001).

All phenolic parameters graphics depict higher values for rose wine without fining than rose wine after fining. For this parameter, differences are even more explicit. The difference can be justified with the quantity of fining agents used, leading to the basic characteristics of the wine affecting colour and, in this case, tanning power.

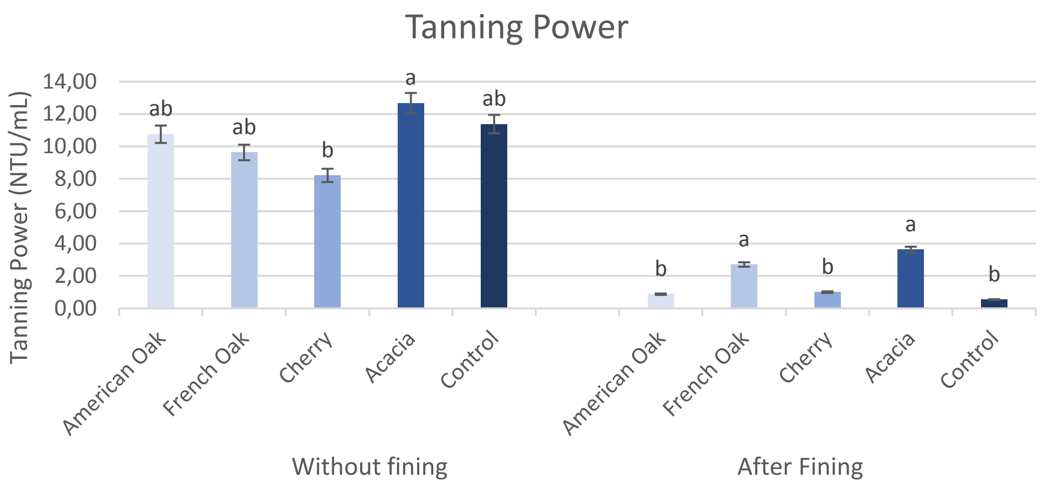

Figure 8 - Concentrations of tanning power after 20 days of storage period of a rose wine (without and after fining) in contact with different wood chips species. Values with different letters indicate that statistically significant differences were detected with Tukey's test (ρ <0,05)

Also in this parameter, rose wine without fining samples treated with oak chips do not differ from control sample, suggesting that no significant impact occurs by the treatment after the 20 storage days.

As in previous observations, also in figure 8, the wine aged with acacia chips showed the highest values during the storage period in both cases, so especially the wine aged in contact with chips from acacia wood suggest more astringency perception when compared with the other wood chip species, even than control wine.

4.3 Colour Parameters

4.3.1 Colour Intensity

By the results presented previously, parameters related with colour are expected to be higher in rose wine without fining, since polyphenolic compounds from wood can also contribute directly or indirectly to colour evolution and stability, influencing the formation of anthocyanin derivatives that takes place during wine ageing (Es-Safi and Cheynier, 2004; Revilla *et al*., 2005). However, it can be seen in figure 9 that the scale of the colour intensity parameter is smaller than all previous ones, meaning that their values have little expression. In this parameter, there are no significance letters on both rose wines before and after fining, meaning there is no differences between them – neither between each wood nor between them and the control wine.

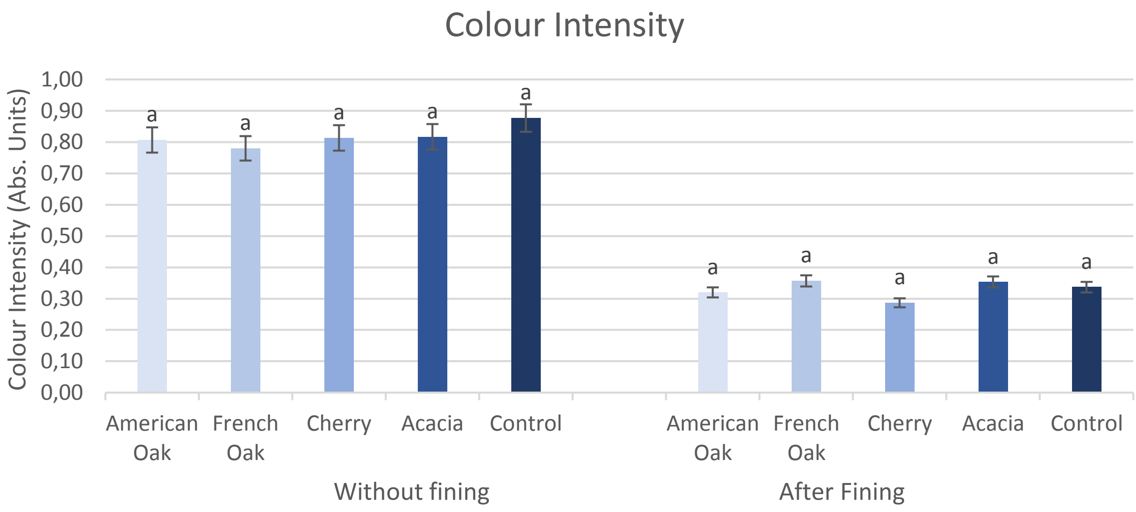

Figure 9 - Concentrations of colour intensity after 20 days of storage period of a rose wine (without and after fining) in contact with different wood chips species. The same significance letter (a) indicates that there were no statistically significant differences detected in analysis of variance (ANOVA)

4.3.2 Colour Hue

It is important to refer that the initial colour (T0) of the samples without and after fining presented very distinct tones: rose wine without fining – light salmon; rose wine after fining – medium pink. Some authors concluded that wine stored in alternative systems (chips and staves) alters its chromatic characteristics more rapidly, while wine treated with chips displayed increased brownish tonalities (Del Alamo Sanza *et al*., 2004b).

In the wines without fining the means do not significantly differ among them and so the Tukey Test was not applied. This is the first parameter where the samples after fining present superior values compared to wines without fining.

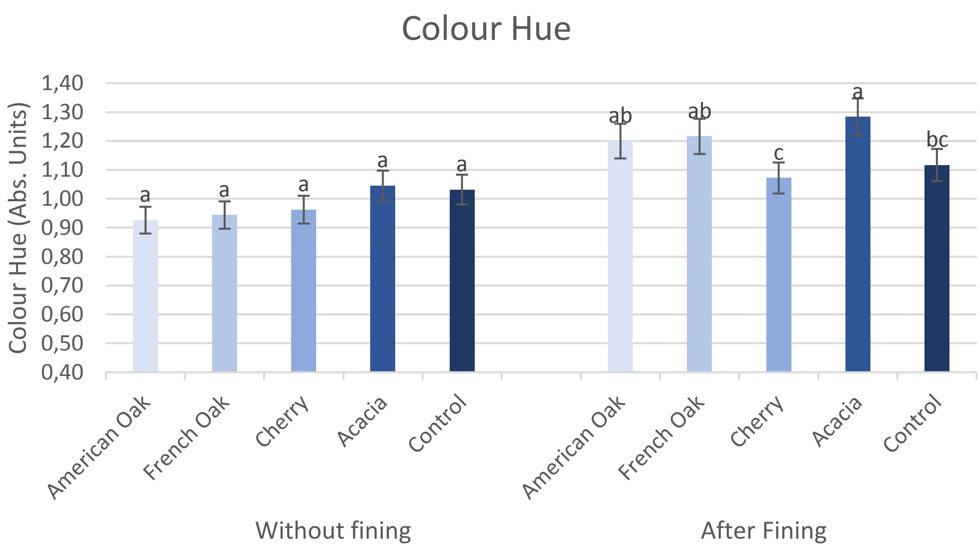

Figure 10 - Concentrations of colour hue after 20 days of storage period of a rose wine (without and after fining) in contact with different wood chips species. Values with different letters indicate that statistically significant differences were detected with Tukey's test (ρ <0,05)

Figure 10 shows that wines aged with cherry wood chips presents the lowest value in rose samples after fining treatments. Once again, wines treated with acacia chips revealed the highest values, while oak wood samples present the same significance letters (ab), that is, they have no differences among them.

Maybe the 20 days of storage weren't enough to cause impact in colour related with control wine and to the different wood species, in the case of the wines without fining.

4.3.3 CIELab Method: Chromatic characteristics

Table 3 - Mean values of CIELab parameters in CIELab units of the rose wines (without fining) aged in contact with different wood chips species after a storage of 20 days.

	CONTROL (W.F)	ACACIA (W.F)	A.OAK (W.F)	CHERRY (W.F)	F.OAK (W.F)
L*	**41,28**	**46,14**	**48,45**	**42,54**	**41,67**
a*	**11,77**	**11,21**	**12,31**	**12,05**	**12,22**
b*	**12,87**	**12,69**	**8,59**	**12,69**	**13,42**
C*	**17,44**	**16,93**	**15,02**	**17,49**	**18,15**

American oak is the sample that shows the highest value of Luminosity (L*) and also tendency towards redness (a*), however French oak presents a quite similar value for the same parameter.

The values of parameter (b*) present a tendency to yellowness, the French oak being the highest yellowness. In this case, American oak presents the lowest value.

Table 4 - Mean values of CIELab parameters in CIELab units of the rose wines (after fining) aged in contact with different wood chips species after a storage of 20 days.

	CONTROL (A.F)	ACACIA (A.F)	A. OAK (A.F)	CHERRY (A.F)	F.OAK (A.F)
L*	**54,20**	**53,95**	**53,25**	**46,76**	**51,79**
a*	**5,03**	**4,98**	**5,49**	**6,69**	**5,73**
b*	**3,54**	**4,57**	**3,24**	**10,36**	**2,70**
C*	**6,15**	**6,76**	**6,37**	**12,33**	**6,33**

Considering after fining samples, the wine treated with cherry wood shows the highest values in all parameters, except in luminosity (*L**) in which control wine is outstanding. The cherry shows a higher tendency towards redness, once has the higher value (*a**) also a tendency to yellowness (*b**). In chroma parameter (*C**) this sample shows a difference from the 4 other samples, because its value is twice.

The CIELab results are merely guides, once there was only one measure.

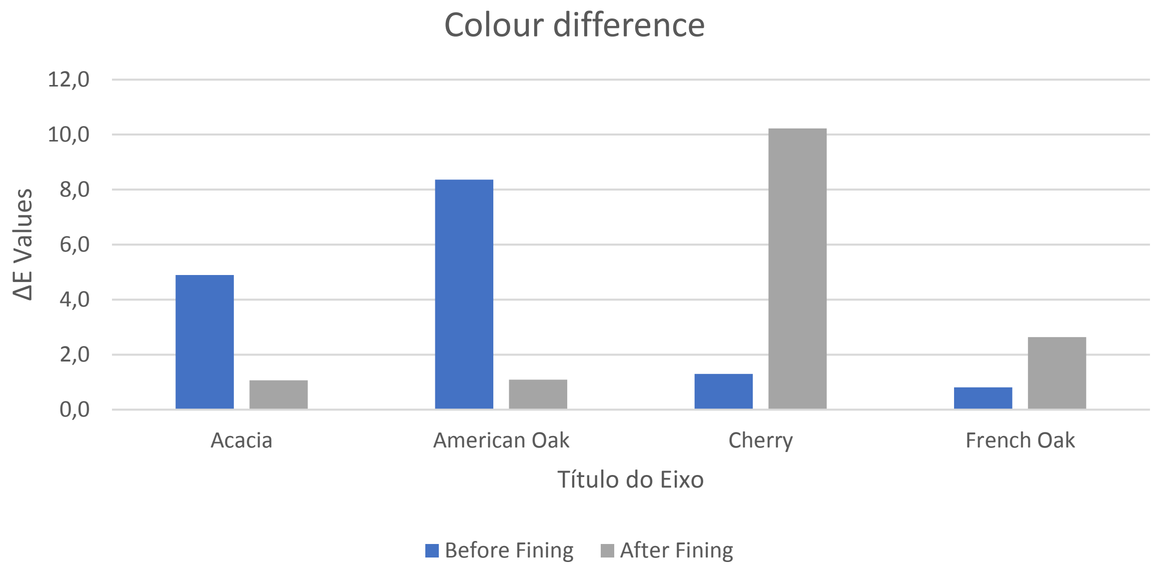

Figure 11 - Mean values of colour differences (ΔE) of the wines (without and after fining) aged in contact with different wood chips species after a storage period of 20 days. Values of ΔE higher than 2 units can be distinguished by human eye

Parameter ΔE is calculated based on the a*, b* and L* values marking the difference between the potential or theoretically perceptible colour during tasting, when the wine is viewed through the glass (Sanchez-Iglesias *et al*., 2009), with limits set by values higher or equal to 2 CIELab units. Considering this limit, some results were obtained:

- For the sample treated with acacia wood differences can be detected for rose wine without fining, but the same doesn't happened for rose wine after fining.
- American oak, reveals a high value for the sample without fining, which permits to detect a quite difference in total colour after the storage period with this wood but for the rose wine after fining the same doesn't occur.
- In cherry wood the situation is quite different: the sample of the wine without fining the presented values cannot be detected by human eye; however, the sample after fining shows ΔE value higher than 2 which allow to be clearly visible the colour difference after 20 storage days in respect of control wine.
- The overall results revealed that French oak detects less differences in final colour. In the wine without fining the difference is below 1 and so impossible to be noticed by the human eye. Considering the sample after fining ΔE is slightly higher than 2, which gives a perception about colour difference.

4.3.4 Total Anthocyanins

The figure 12 shows the relation between two samples of wines and the content of total anthocyanins (all the values are expressed in mg/L malvidin-3-monoglucoside) during the 20 storage days.

In the case of rose wines without fining, American and French oak present lower values and the same significance letter (c) while the sample treated with acacia wood is outstanding when compared with other wines without fining. This means a strong difference when comparing with oak and cherry woods (superior to 40 mg/L). This phenomenon can be explained by intrinsic characteristics of the acacia wood acacia, namely some phenolic compounds – non-flavonoid phenols which previously oxidize protecting the monomeric anthocyanins. And so, this parameter presents higher values when compared to other samples. On the other hand, the wines treated with cherry wood chips present intermediate values between acacia and oak woods.

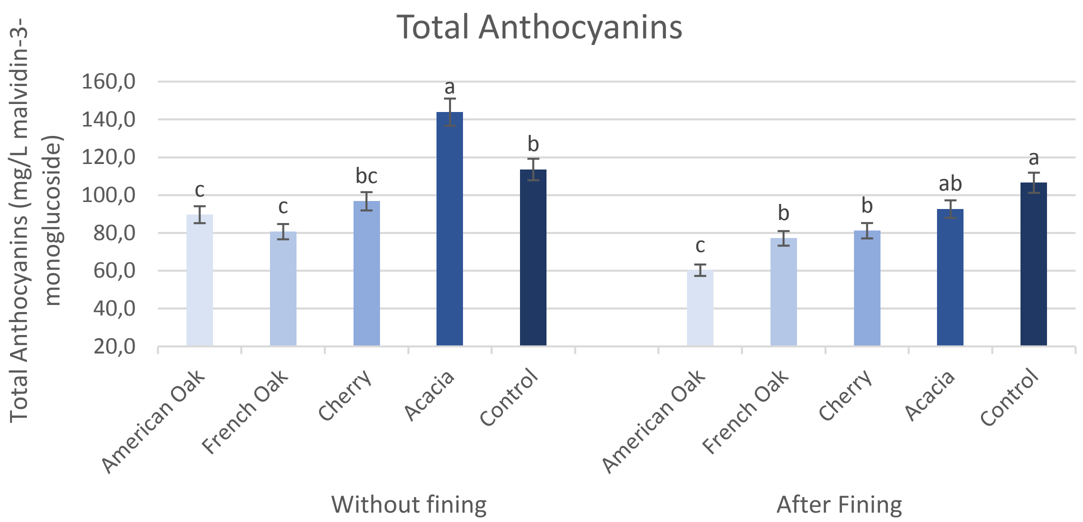

Figure 12 - Concentrations of total anthocyanins after 20 days of storage period of a rose wine (without and after fining) in contact with different wood chips species. Values with different letters indicate that statistically significant differences were detected with Tukey's test (ρ <0,05)

Considering wines after fining there are some similarity when compared with wines without fining, although those values show less concentration differences, which are below 20 mg/L. This is expectable once after fining treatments the colour of the wine can be removed. Although presenting different significance letters, oak woods show the lowest values in both situations, which suggest that anthocyanins are the phenolic compounds which are the first to oxidize. The wines treated with French oak and cherry woods are not significantly different.

Control wine is the sample with the highest level in total anthocyanins (more than 100 mg/L). This is the opposite of what has been observed in most cases: wines treated with acacia chips presents higher values.

4.3.5 Ionized Anthocyanins

There are different anthocyanins types. The ionized anthocyanins are responsible for the colour of the wine. This parameter reveals very low concentrations and few variations in both situations.

In the first case, wines without fining, the samples treated with oak and acacia wood chips do not show statistically significant differences between them. However, cherry wood differs from the other samples (control wine, acacia and oak) showed the lowest value in this parameter, less than 2 mg/L. In this specific situation, control wine stands out comparing to other samples, presenting a value close to 3 mg/L, which allows to conclude that time/amount of added wood was not enough to obtain a difference when compared to control wine.

For wines after fining, as is shown in the figure 13, the samples have the same significance letter (a), meaning that the Tukey Test is not applied. However, is still possible to observe that the obtained values correspond to very low concentrations, under 2 mg/L. The sample, which slightly stands out from the others, is the wine treated with French oak wood chips.

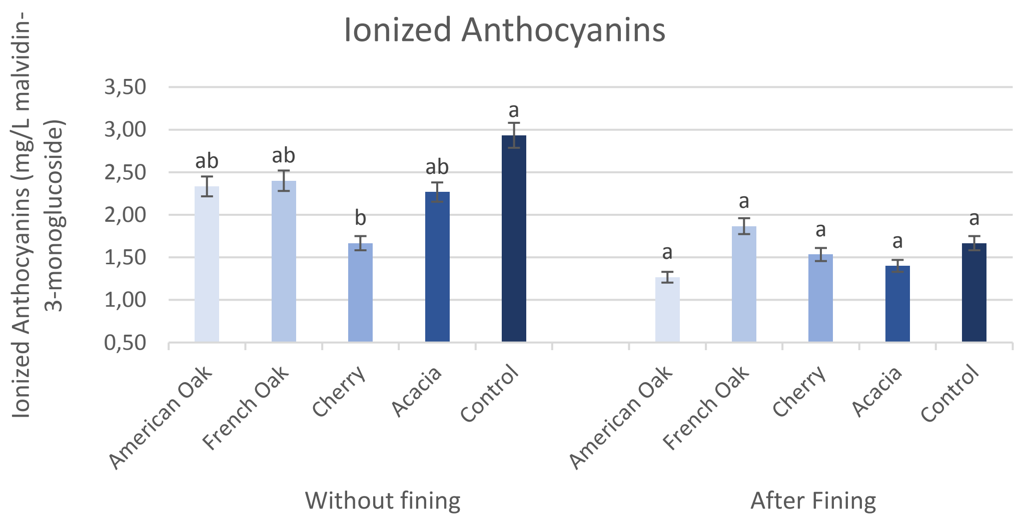

Figure 13 - Concentrations of ionized anthocyanins after 20 days of storage period of a rose wine (without and after fining) in contact with different wood chips species. Values with different letters indicate that statistically significant differences were detected with Tukey's test (ρ<0,05)

4.3.6 Degree of Ionized Anthocyanins

The figure 14 shows the % degree of ionized anthocyanins. Colour stability during ageing closely corresponds to the degree of polymerization between anthocyanins and other phenolics (Auw *et al.,* 1996).

Likewise the previous graphic, also in this case can be observed that all the values are very low, under 5%.

In general, rose wines without fining present higher values than rose wines after fining, but also more differences between them. The wine treated with French oak shows the higher % of ionized anthocyanins while the wines treated with acacia and cherry wood chips have the same significance letter (b) and their value is lower than control wine.

The sample treated with French oak wood presented the higher value. This sample stands out also in wines after fining, even if they have not statistically different values - that's why they got the same significance letter (a).

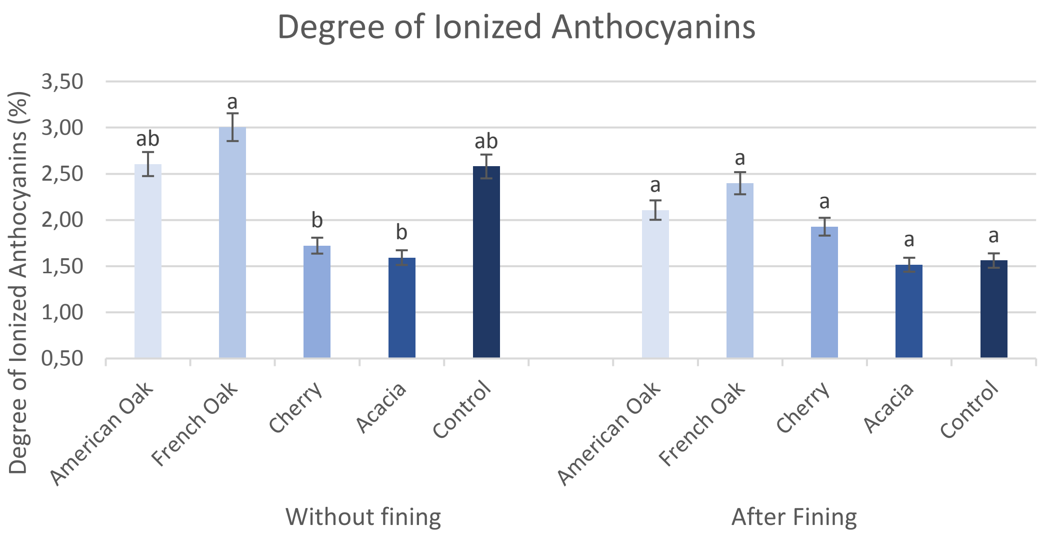

Figure 14 - Values of degree of ionized anthocyanins after 20 days of storage period of a rose wine (without and after fining) in contact with different wood chips species. Values with different letters indicate that statistically significant differences were detected with Tukey's test (ρ<0,05)

4.3.7 Total Pigments

The total pigments resulting in sum of phenolic compounds (such as flavonoids), anthocyanins and associations between them. Figure 15, shown the total pigments quantified in different rose wine samples aged in contact with different wood chip species after 20 storage days.

The sample treated with acacia wood chips reveals the highest value in the case of rose wine without fining. This result was expectable after the observation and conclusion in flavonoid phenols and total anthocyanins parameters, as they are related.

The same happens in other samples (American oak, French oak, cherry and control wine). Considering rose wines after fining is also possible to establish a relation with total anthocyanins parameter meaning the values are very similar among them.

The wines treated with cherry and French oak wood have the same significance letter (b), whereas the wine treated with acacia wood reveals a light superiority when related to wines treated with the other three woods. Its value only stays below the control. In spite of being slight, the control wine presents the highest value.

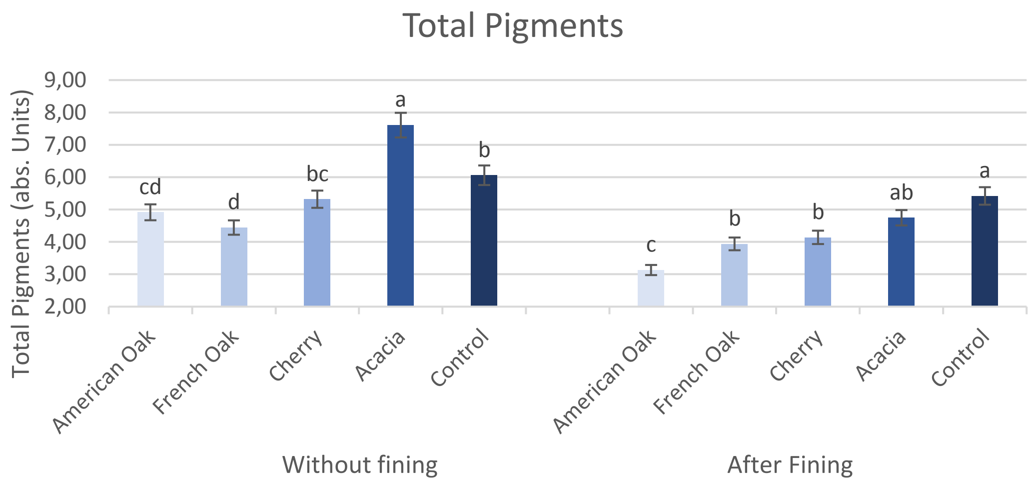

Figure 15 - Concentrations of total pigments after 20 days of storage period of a rose wine (without and after fining) in contact with different wood chips species. Values with different letters indicate that statistically significant differences were detected with Tukey's test (ρ<0,05)

When compared to flavonoid phenols parameter, the relation is more evident in case of wines after fining than wines without fining.

4.3.8 Polymeric Pigments

During the ageing process, anthocyanins undergo a series of processes, which increase the polymeric pigments content and consequently decrease monomeric anthocyanins (Ribéreau-Gayón *et al*., 2003), thus contributing to an increase in the colour tonality and intensity, along with colour stability (Pérez-Magariño *et al*., 2004).

The polymeric pigments are essentially polymerized anthocyanins and polymers of anthocyanins and condensed tannins.

This is a parameter directly related with colour parameters: colour intensity and total anthocyanins. So, similarly to the first (colour intensity) the polymeric pigments also do not present significantly different means among the samples for none of the analysed situations – rose without and after fining, that's why none of the above graphics show significance letters. The measure units are the same for the 2 parameters, however the scale is slightly different: polymeric pigments show for both cases values below 0,3 while colour intensity parameter only present those values in case of wines after fining.

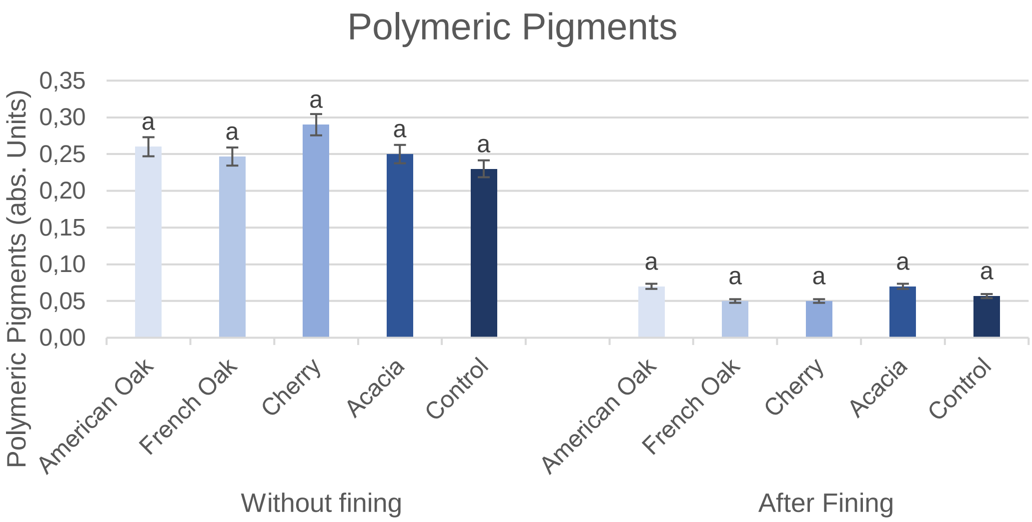

Figure 16 - Concentrations of polymeric pigments after 20 days of storage period of a rose wine (without and after fining) in contact with different wood chips species. The same significance letters (a) indicates that there were no statistically significant differences detected in analysis of variance (ANOVA)

It's important to emphazise that the use of different botanic chip species didn't result in a significant change in the polymeric pigments of the wines. There is no difference when the presence of chips were used in wines without or after fining.

In the case of rose wines without fining, the wine treated with cherry wood chips present the highest value (0,29 abs. units) followed by the samples of American oak, acacia and French oak.

During the same experiment, it was possible to observe the low values of the wine samples after fining. The highest value found was obtained by the wines treated with American and acacia wood under 0,1.

Comparing the parameters: polymeric pigments with the already analysed – total anthocyanins there are some differences between them. In this case, for wines without fining, cherry wood sample shows the highest value followed by American oak and acacia wood.

Considering rose wines after fining the differences are less instead of the very low values presented in the polymeric pigments (figure 16). American oak and acacia samples have the same values while French oak and cherry wood show lower values but no differences between them.

4.3.9 Polymerization Index

Polymerization is important in stabilizing wine colour by protecting the anthocyanidin molecule from oxidation or other chemical modifications (Jackson, 2000), therefore these compounds are less susceptible to lack of colour.

In general, this index has very low % with all values below 7%. They present different means among them and also with different significance letters.

In the case of wines without fining the sample treated with French oak and cherry wood reveal a slight superiority in % of polymerization index. This allows to conclude that it is less prone to loss of colour than the other samples.

In this parameter, acacia wood differently from the usual results, has the lower %, meaning that is more susceptible to loss of colour. Although it has a high content of total anthocyanins, it has a low polymerization index, which can be explained by intrinsic characteristics of acacia wood, which does not present the tannins and anthocyanins in a combined form. Sanz *et al*., (2010) described that tannins were also different in acacia wood, showing only a small amount of condensed tannins.

Considering the wines after fining, the % of the samples are all very low – values between 1 and 2%. The means doesn't show statistically significant differences among them, so the Tukey Test was not applied. Apparently, these samples are more subject to the loss of colour, oxidation and other chemical changes, once their polymerization index is very low. This low value of the polymerization index is also dependent on the removal of phenols that occurs after fining.

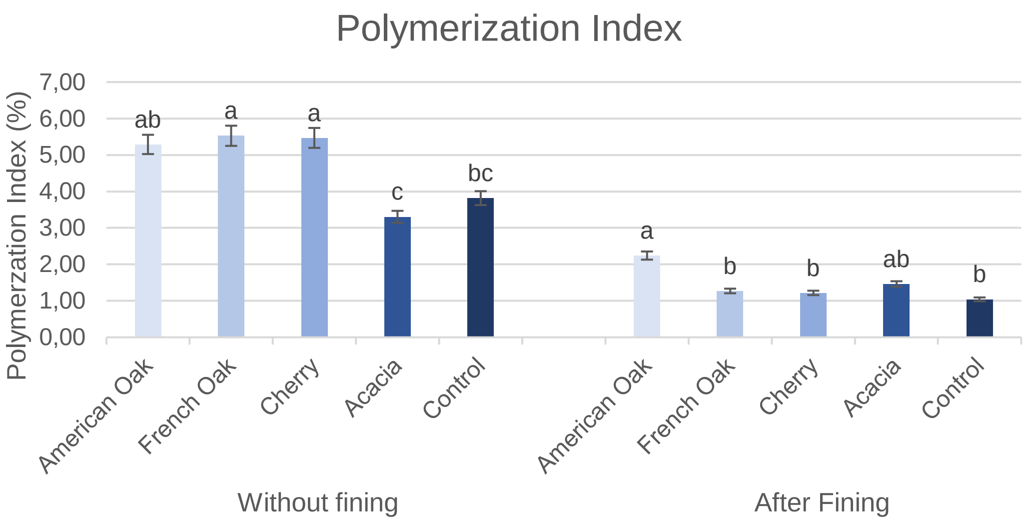

Figure 17– Values of polymerization index after 20 days of storage period of a rose wine (without and after fining) in contact with different wood chips species. Values with different letters indicate that statistically significant differences were detected with Tukey's test (ρ<0,05)

4.4 Sensorial Characteristics

A sensory analysis was conducted for the wines (without and after fining) that had been ageing in contact with different wood chips species. Tasting was divided in 4 steps, with the tasters giving scores to visual evaluation, aroma and taste attributes and to global appreciation. The results obtained for the different aspects in the wines without fining are summarized in table 5.

Table 5: Results obtained in the sensory analysis for the wines without fining, presented as mean values (standard error between brackets).

		Control	**American Oak**	**French Oak**	**Cherry**	**Acacia**
	Attributes	Mean Value	Mean Value	Mean Value	Mean Value	Mean Value
Visual	Colour intensity	3.8(0.31)	3.2(0.48)	3.2(0.31)	3.2(0.31)	3.1(0.22)
	Limpidity	3.8(0.48)	4.2(0.40)	3.8(0.40)	4.2(0.31)	2.9(0.67)
Aroma	Intensity	3.5(0.43)	3.0(0.45)	3.3(0.33)	3.3(0.33)	3.1(0.33)
	Persistency	3.3(0.33)	2.3(0.33)	3.0(0.26)	3.2(0.31)	2.9(0.33)
	Quality	3.3(0.33)	2.5(0.34)	3.0(0.26)	3.5(0.34)	3.3(0.33)
	Red Fruits	3.3(0.21)	2.3(0.33)	3.0(0.52)	2.5(0.43)	2.5(0.17)
	Woody	2.8(0.31)	3.5(0.50)	3.0(0.52)	3.2(0.48)	2.3(0.43)
	Floral	2.7(0.42)	2.2(0.48)	2.8(0.48)	2.8(0.40)	2.5(0.33)
	Vegetal	2.7(0.21)	2.8(0.40)	2.2(0.54)	2.8(0.48)	2.2(0.42)
	Equilibrium	3.7(0.42)	2.8(0.31)	3.2(0.17)	3.7(0.21)	2.6(0.45)
Taste	Acidity	2.7(0.49)	3.3(0.33)	3.3(0.33)	3.2(0.31)	2.9(0.43)
	Sweetness	2.5(0.22)	2.7(0.33)	2.5(0.22)	2.7(0.49)	1.7(0.31)
	Bitterness	3.3(0.56)	2.8(0.60)	3.3(0.42)	2.5(0.43)	2.7(0.37)
	Persistency	3.5(0.43)	3.3(0.21)	3.2(0.48)	3.5(0.22)	2.8(0.33)
	Astringency	3.0(0.26)	3.3(0.49)	3.5(0.34)	3.7(0.33)	3.0(0.31)
	Equilibrium	3.8(0.17)	3.5(0.34)	3.0(0.26)	3.3(0.49)	2.9(0.33)
Global Appreciation		3.3(0.21)	2.7(0.42)	2.2(0.17)	4.0(0.37)	2.8(0.21)

The results obtained in the sensory analysis for the different attributes in the wines after fining are summarized in table 6.

Table 6: Results obtained in the sensory analysis for the wines after fining, presented as mean values (standard error between brackets).

		Control	American Oak	French Oak	Cherry	Acacia
	Attributes	Mean Value	Mean Value	Mean Value	Mean Value	Mean Value
Visual	Colour intensity	3.1(0.44)	3.9(0.30)	3.8(0.37)	3.4(0.38)	3.9(0.30)
	Limpidity	4.6(0.18)	4.1(0.48)	4.4(0.38)	4.4(0.26)	4.8(0.16)
Aroma	Intensity	3.5(0.33)	3.8(0.31)	3.4(0.32)	2.8(0.25)	2.5(0.27)
	Persistency	3.0(0.19)	3.8(0.25)	3.1(0.30)	2.8(0.25)	2.8(0.41)
	Quality	2.8(0.16)	3.6(0.18)	2.9(0.48)	3.4(0.26)	3.1(0.40)
	Red Fruits	2.3(0.37)	2.4(0.26)	2.0(0.38)	2.8(0.25)	2.5(0.46)
	Woody	2.3(0.49)	2.9(0.35)	3.3(0.49)	2.4(0.26)	2.3(0.37)
	Floral	2.8(0.31)	2.9(0.23)	2.6(0.46)	3.0(0.19)	2.1(0.23)
	Vegetal	2.6(0.32)	2.3(0.45)	2.3(0.31)	2.6(0.18)	2.3(0.25)
	Equilibrium	2.8(0.31)	3.3(0.31)	2.4(0.32)	3.0(0.27)	3.0(0.38)
Taste	Acidity	3.5(0.27)	3.1(0.30)	2.6(0.18)	2.6(0.38)	3.1(0.30)
	Sweetness	2.5(0.27)	2.5(0.38)	2.3(0.31)	2.5(0.33)	2.3(0.49)
	Bitterness	2.6(0.32)	2.8(0.41)	2.6(0.42)	2.3(0.25)	2.8(0.45)
	Persistency	3.1(0.13)	3.3(0.37)	2.6(0.26)	2.6(0.26)	2.3(0.41)
	Astringency	2.8(0.31)	2.6(0.26)	2.4(0.26)	2.5(0.19)	3.0(0.38)
	Equilibrium	3.1(0.23)	2.6(0.18)	2.8(0.25)	3.4(0.26)	3.0(0.33)
Global Appreciation		3.4(0.32)	3.0(0.19)	2.6(0.38)	3.3(0.16)	3.3(0.45)

Scores given by the tasters to visual aspect, namely limpidity and colour intensity, were analysed by ANOVA (α=0.05), and no significant differences could be associated to the different wood species, both in wines without fining and after fining. Concerning aroma parameters, ANOVA was also conducted to assess the influence of the different wood species in the scores given to intensity, persistency, quality, red fruits, woody, floral, vegetal and equilibrium. Taste parameters were analysed similarly towards the marks given to acidity, sweetness, bitterness, persistency, astringency and equilibrium. Global appreciation was scored as a separated parameter. For the wines without fining, no significant differences (α=0.05) were found between woody species regarding aroma and taste parameters. Concerning the global evaluation, the ANOVA revealed significant differences between the woody species (Table 7), with cherry wood being significantly better evaluated than American and French oak. Acacia wood and control showed an intermediate behaviour.

Table 7 Mean scores of global appreciation given by the tasters to the wines without fining aged in the different woody species.

Woody species	Mean value of global appreciation[1]
Cherry	4.000000 a
Acacia	3.333333 ab
Control	3.333333 ab
American Oak	2.666667 b
French Oak	2.166667 b

[1]Different letters in column correspond to significantly different mean values, according to Tukey test (α=0.05).

In rose wine after fining, significant differences were observed only in the intensity of the aroma (table 8). American oak contributed to the highest aroma intensity, in opposition to acacia wood, that contributed to the lowest. No other significant differences related to wood species were identified in other aroma or taste parameters, neither in global appreciation.

Table 8: Mean scores of aroma intensity given by the tasters to the wines after fining aged in the different woody species

Woody species	Mean value of intensity[1]
Cherry	2.750 ab
Acacia	2.500 b
Control	3.500 ab
American Oak	3.750 a
French Oak	3.375 ab

[1]Different letters in column correspond to significantly different mean values, according to Tukey test (α=0.05).

5. CONCLUSIONS

In this study the aim was to get, as much as possible, the knowledge of the ageing process of rose wines in the presence of wood chips, measuring the impact of different wood species, with the same toasting level in two samples from the same rose wine: before and after fining.

- Acacia wood revealed the higher values for the majority of the parameters. This happened in the wine without fining as well as after fining. However, none of the samples got the preference in sensorial global appreciation.
- The quantity of wood chips used in this experiment associated to 20 storage days wasn't enough to obtain conclusive results, mainly in the rose wines after fining – in the following parameters: colour intensity; ionized anthocyanins; degree of ionized anthocyanins and polymeric pigments. The values obtained were very low and the analysis of variance through ANOVA method didn't show statistically significant differences.
- The wines without fining in some parameters didn't present results for the Tukey Test (graphics without significance letters), but the majority show considerably higher values than the wines after fining. This situation may be explained by the use of fining agents which influences the phenolic matrix of the rose wine.
- For the sensory analysis, the samples aged in contact with cherry wood chips – for the wines without fining and control wine (without wood chips) – for the wines after fining, obtained a higher punctuation on the global appreciation parameter.
- The application of parameters created for red wines brings some difficulties in the analysis/definition of the results once the phenolic composition of a rose wine differs from the phenolic composition of a red wine especially in the parameters related with colour and anthocyanins.
- The results showed are not definitive, as several factors remain unknown such as the influence of wood concentration, aged time, interaction between phenolic compounds from wine and compounds extractable from wood and the relation between the chemical variation and sensory response. Thus, further work about this subject ought to be continued.

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APPENDIX

Ficha de Prova – Vinhos Rosés

Provador:____ Data:___________________

Escala: 1 (menos intenso) a 5 (mais intenso)

Amostras de vinho									
Aspeto									
Intensidade da Cor									
Limpidez									
Aroma									
Intensidade									
Persistência do aroma									
Qualidade do aroma									
Frutos vermelhos									
Madeira									
Floral									
Vegetal									
Equilíbrio									
Sabor									
Acidez									
Doce									
Amargo									
Persistência									
Adstringência									
Equilíbrio									
Apreciação Global									

Observações:

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